HUMAN AMNIOTIC MEMBRANE AND *CURCUMA LONGA*–DERIVED GEL FOR THE TREATMENT OF BURN INJURIES IN A RAT MODEL

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Hafizur Rahman[1, #], Ayesha Siddika[2, #], Polash Chandra Karmakar[2, #], Tusher- Al-Arafat[3, #], Liakat Hossain[2], Hasib Adnan[2], Farha Matin Juliana[1], Naznin Akhtar[2] and S. M. Asaduzzaman[2, *]

[1]*Department of Biochemistry and Molecular Biology, Jahangirnagar University, Savar, Dhaka 1342, Bangladesh*
[2]*Institute of Tissue Banking and Biomaterial Research, Atomic Energy Research Establishment (AERE), Savar, Dhaka 1349, Bangladesh*
[3]*Department of Microbiology, Jashore University of Science and Technology, Jashore 7408, Bangladesh*

[#] These authors contributed equally to this work

ABSTRACT

Purpose: Burn injuries are a serious medical issue worldwide. In this study, *Curcuma longa* (CUR) loaded human amniotic membrane (HAM) gel was formulated to find an effective treatment for burn wounds with a faster healing rate and cost-effectiveness. **Methodology**: Physicochemical evaluation of the gel formulations was carried out through visual examination, assessing factors such as consistency, homogeneity, pH, FTIR, NMR analysis, and brine shrimp lethality test. To determine the burn wound healing efficacy, 20 female Wistar rats with 150-180g body weight were randomly divided into five groups: negative control, HAM, CUR, HAM+CUR and positive control. Burns were created and then treated with respective gels. **Major findings**: All gel formulations were essentially homogeneous and had good consistency with a pH between 6.68 and 6.80. FTIR and NMR studies supported the presence of collagen and skin irritation study displayed no edema or erythema after 7 days of topical application. After 16 days of treatment, the HAM+CUR group showed superiority (94.31±2.24 %) in burn wound healing, which was statistically the most significant compared to the control group ($P<0.001$). In addition, it takes only 19.67±1.52 days for the HAM+CUR group for re-epithelialization, while HAM, CUR, positive and the negative control group required 25.33±1.53, 24.67±2.08, 22.0±2, and 31.67 ±1.15 days for re-epithelialization, respectively. Histological evaluation revealed that HAM+CUR treated rat groups had significant epidermal regeneration, granulation tissue formation and collagen deposition. **Conclusions:** Therefore, HAM+CUR gel demonstrates significant potential therapeutic agent for treating burn wounds.

KEYWORDS: Human Amniotic Membrane, *Curcuma longa*, Burn wounds, Wound contraction, Epidermal regeneration

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***CORRESPONDING AUTHORS: S. M. Asaduzzaman, Institute of Tissue Banking and Biomaterial Research, Atomic Energy Research Establishment (AERE), Savar, Dhaka 1349, Bangladesh**
Email: sikderasad@yahoo.com

Introduction

Burning is one of the most serious injuries and has remarkable physiological and psychological consequences for the patient (Prasanna et al., 2004). Burned skin is more susceptible to infections leading to systemic sepsis and severe septicemia and increases the number of deaths and disabilities in the world (Xue et al., 2013). However, exponential growing in biomedical research related to the treatment of burns, mortality from burns has decreased (Emiroglu et al., 2017). Various synthetic products are used in wound treatment, but they have limitations such as complications and side effects (Kaddoura et al., 2017). Therefore, scientists are working to develop gels based on biomaterials as a sustainable alternative to conventional antibiotics or wound dressings.

Human amniotic membrane (HAM) has been recognized by physicians as an effective means of wound healing. The HAM graft has been widely used for wound coverage, ocular surface diseases, diabetic ulcers, and various types of postoperative and post-traumatic wound injuries (Islam et al., 2023). The reduction of scars after the application of HAM to wounds due to anti-inflammatory effects, acceleration of epithelial cells, and suppression of fibrosis. HAM expresses several neurotrophic and angiogenic factors: endothelin 2 and 3, vascular endothelial growth factor, vascular endothelial growth factor B, ephrin receptor A2, B1, B3, B4, B5, neuropilin-2, nerve growth factor receptors, Semaphorin-F19 and erythropoietin and its receptors that promote wound healing (Jhumi et al., 2023). Previous studies have shown that a gel prepared from HAM and titanium

dioxide nanoparticles significantly promoted wound healing in Wistar rats (Islam et al., 2023). The standard treatment for burn wounds currently practiced clinically is HAM allograft (Akter et al., 2025).

Curcuma longa (CUR) is considered a promising wound-healing agent (Hao et al., 2000). It has been observed that CUR enhances wound healing in various animal models. In experimental rats, CUR put into stable nano gel demonstrated safe and regulated skin permeability along with encouraging cutaneous wound healing and anti-inflammatory properties (Adamczak et al., 2020). In previous studies on full-thickness wounds in the rat model, the application of Cur-loaded chitosan/poly (ethylene oxide)/collagen nanofibers indicated significant improvement in the mean wound area closure (Jirofti et al., 2021). By boosting the production of extracellular matrix (ECM) proteins and transforming growth factor (TGF)-β1, CUR facilitates the healing of wounds by increasing the amount of granulation tissue (Sidhu et al., 1998; Panchatcharam et al., 2006). Furthermore, free radicals can be effectively neutralized by CUR, which lowers the activity of antioxidant enzymes and the inflammatory response (Sidhu et al., 1999). CUR also inhibits the oxidative damage caused by peroxide in human keratinocytes and fibroblasts by scavenging free radicals and encouraging the downregulation of the PI3K/AKT/NFκB pathway (Phan et al., 2001). CUR also have anti-infective and antioxidant qualities, according to earlier research (Mohanty et al., 2012; Dimas et al., 2015).

In this study, we aimed to formulate a unique formulation by mixing HAM powder with traditional CUR powder and investigating their wound-healing efficacy in second-degree burns in a rat model by evaluating various macroscopic and histological parameters.

Methods

Experimental design

This study was conducted to find out the effectiveness of CUR and HAM gels to speed up the healing process of burn wounds in a Wistar rat model. This study included multiple steps: (i) a mixture of HAM extract and CUR was made, and research on skin irritation and physical attributes such as homogeneity, pH, FTIR, and NMR was carried out. (ii) inducing burn injuries in models of Wistar rats. (iii) rats were used in an in vivo wound healing experiment to examine the wound healing potential of a gel generated from HAM extract and CUR. The wound healing duration was observed in the injured area, and the percentage of wound contraction was calculated.

Preparation of HAM powder

Human amniotic sacs were obtained from 10±2 cesarean section patients who tested negative for hepatitis B, hepatitis C, syphilis, and HIV infections which was approximately 300±20g. Shortly after being collected, HAMs were separated carefully from the chorion by using sterile forceps. After that, HAMs were repeatedly cleaned using physiological brine saline (0.85% NaCl). Following cleaning, the HAM was exposed to a 50 µg/mL solution of streptomycin and a 50 µg/mL solution of penicillin for 15 minutes. Membranes were then frozen for a whole day at a temperature lower than zero. After that, membranes were freeze-dried for a full day at -55°C. Gamma radiation at a dose of 25 kGy was then used to sterilize the freeze-dried HAM. Ultimately, a blender was used to chop the irradiated membranes into tiny bits and powder them. After that, the powdered HAM weighing about 25±5g was kept in the refrigerator until further use (Islam et al. 2023).

Preparation of CUR powder

Mature CUR was collected from a local market, Baipail Vegetables Market Savar, Daka-1349, Bangladesh. CUR was then placed in a beaker and cleaned with tap water. After that, CUR was rinsed for five minutes in 1% NaCl, 70% ethanol solution, and distilled water (DW), respectively. Lastly, CUR was placed in a beaker and briefly drained in sunlight. After that, it was shade-dried for at least four days to develop crispy. Following the use of an electric grinder to convert the crumpled CUR into a powder, the powder was weighed and kept at -40ºC.

Formulation of HAM gel, CUR gel and CUR incorporated HAM gel

The HAM and CUR powder were combined to prepare gel according to the method of Jhumi et al., 2023. The following procedure was used to make gel by combining the powders of HAM and CUR. Three different kinds of gel formulations were made in all. (i) HAM gel: 5g of powdered HAM, 3.70g of CMC-Na (Loba Chemie, India), 1.25g of propylparaben (SUPELCO-Sigma Aldrich, Germany), 20 mL of glycerin (CP, China), 20 µL of triethanolamine (Merck, Germany), and Up to 100 mL of DW, (ii) CUR gel: 50 mL glycerin, 0.5 µL triethanolamine, 0.1 g propylparaben, 5g CUR powder, 4.5g CMC-Na, and DW up to 100 mL, (iii) HAM+CUR gel: 0.5 µL triethanolamine, 30 mL glycerin, 4.5 g CMC-Na, 0.1 g propylparaben, 5 g CUR and 5 g HAM powder, and DW up to 100 mL. First, autoclaved distilled water was added to a beaker to prepare a gel. Glycerine was then added to the beaker while it was continuously stirred. After that, the solution was supplemented with CUR and HAM powder at a 1:1 ratio. Subsequently, a magnetic stirrer (Phoenix Instrument, RSM-0.2HD) was used to constantly add and dissolve precisely weighed CMC-Na in the solution for 30 minutes at 1200 rpm and 30 ºC. Next, propylparaben was added and thoroughly combined. Lastly, the mixture was neutralized by gradually adding triethanolamine drop by drop. The mixture kept going until a clear gel formed.

Physicochemical characterization of the formulated gel

Visual examination

The color, consistency and uniformity of the prepared gel formulations were inspected visually. All formulated gels were taken in a container to visualize clearly and also tested their appearance if there were any aggregates.

pH determination

One gram of each gel formulation (HAM, CUR, and HAM+CUR) was combined with twenty-five milliliters of distilled water. A pH meter (HI-98107, Hanna Instruments, Italy) was calibrated with buffer solutions 4.0, 7.0, and 9.0 before being used to measure the mixture's pH. Three duplicates of each experiment were carried out, and average values were determined.

Fourier Transform Infrared Spectroscopy (FTIR) analysis

Samples were put straight into a sample holder (Model: IRprestige21, Shimadzu, Japan) to get FTIR spectra. A Shimadzu FT-IR 8400S Spectrophotometer (Shimadzu Corporation, Japan) was used to record FTIR spectra, with a range of 4000-7000cm-1, resolution of 4cm-1, and number of scans of 20x.

Nuclear magnetic resonance (NMR) analysis

NMR experiments were performed in the 54 mm room temperature bore of a Bruker 7.05 T (300 MHz)

superconducting magnet. All experiments employing the single-chip probe were performed with a repetition time of 2 s, a π/2 pulse length of 2.5 μs, and an acquisition time of 400 ms. The time domain data were post-processed by applying an exponential filter with a decay of 50 ms.

In vitro cytotoxicity test (Brine shrimp lethality test) and In vitro antimicrobial examination of the gel formulation

An *in vitro* cytotoxicity test of gels was accomplished using the brine shrimp (*Artemia salina*) nauplii lethality bioassay method as described by Karmakar et al. (2023). Here, 0.50 mg/ml of vincristine sulfate (Sigma Aldrich Co., St. Louis, MO, USA) was considered as a positive control and seawater without additions was used as a negative control (-Al-Arafat et al., 2023). Antimicrobial activity of gel prepared gel formulations was determined against *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922). This test was performed using disc diffusion antibiotic sensitivity technique (Jhumi et al., 2023). The inhibition zone diameters (mm) around each disc were measured and each assay was performed in triplicate.

In vivo Skin irritation studies

Skin irritation studies were performed on female Wistar rats. The intact skin was used by removing dorsal hairs using an electrical shaver. 12 animals were taken and randomly assigned into three groups (HAM, CUR, HAM+CUR). The gels containing extracts were applied on the skin of animal once a day. The animals were treated daily up to seven days and finally the treated skin was visually examined for erythema and edema.

Assessment of in vivo burn wound healing

Induction of artificial burn wounds in wistar rat models

In total, 20 healthy female Wistar rats of 150–180 g body weight were used in this study and divided into 5 experimental groups: (i) positive control (ii) negative control (iii) treated with HAM gel (iv) treated with CUR gel, and (v) treated with HAM+CUR gel. All animals received human care according to the National Institutes of Health's guideline for the care and use of laboratory animals. The rats were fed normal rat chow and tap water ad libitum. Rats were maintained in the animal quarter at a temperature of 23±2°C, a humidity of 50–55%, and a light/dark cycle of 14 hours/10 hours. Each rat was anesthetized with a 100 mg/kg body weight Ketamine HCl solution (Gonoshasthaya Pharmaceuticals Ltd, Bangladesh) by intraperitoneally administration. The hair in the dorsal area was then cut with an electric hair clipper (KM-3909, Kemei, India) and then shaved with sharp blade. The shaved areas were cleansed with alcohol swab. Burn wounds were created by heated aluminum disc (2.5cm) kept in water bath for 5 minutes at 100ºC which was applied for 15 seconds on the shaved area gently (Islam et al., 2023). The rats were treated with 1 ml of the distinct gel for 16 days, and applied twice topically daily. Gross changes in the wounds were measured from day 0 to day 16. Using a Canon IXUS 130 camera, morphological evaluations, such as wound appearances, were noted and photos were taken.

Measurement of wound contraction

To monitor the contraction of the wound, gradual changes in the wound area were strictly followed. The wound edge was drawn using a millimeter measuring scale at 4-day intervals, with measurements continuing for up to 16 days. The healed area was determined by subtracting the initial wound area from the unhealed region every four days (Hemida et al., 2022). Wound areas were calculated on each day and by using the following formula,

% Wound Contraction= [(Initial Wound Area – Final Wound Area) / Initial Wound Area] × 100

Epithelialization period

Epithelialization was measured by the presence of a matte-appearing epithelial coating distinct from the wound area (Akter et al., 2025). The onset of epithelialization was monitored, and the number of days required for the epithelization within the wound area was noted.

Histological analysis

Histopathological analysis of the tissue samples was performed as described in the literature with minimal modification (Banchroft et al., 1996). Skin specimens from each group (Negative control, HAM, CUR, HAM+CUR, Positive control) were collected on the 4th, 10th and 16th day after burn induction. Skin biopsies were embedded in paraffin blocks overnight in 10% formalin after fixation. Embedded skin tissues were cut into 5μm thick sections using a microtome (OSK 9770 LTRM, Japan) and collected on glass slides. Afterwards the tissue sections were deparaffinized and stained with hematoxylin and eosin stain (H&E). The stained histological sections were examined and evaluated in random order. Images were taken with LEICA DM 500 light electric microscope.

Statistical Analysis

All quantitative data were presented and mean, standard deviations (SD) were calculated by Student's t-test. $P < 0.05$ was considered statistically significant.

Results

Characterization of the gel formulations

Prepared formulated gels had a good consistency and were homogenous. The colors of HAM, CUR, and HAM+CUR gels were off-white, yellow, and light-yellow respectively (Figure 1).

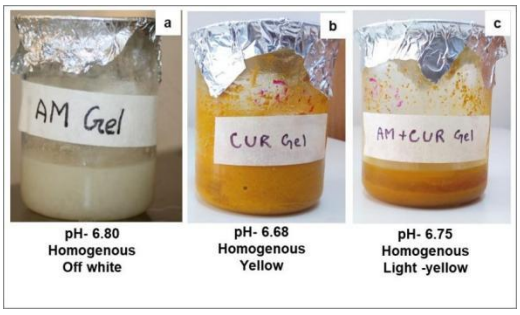

Figure 1. Physical appearance of the gel with pH; **(a)** HAM gel, **(b)** CUR gel, **(c)** HAM+CUR gel

The gels had a pH between 6.68 to 6.80, indicating that they were appropriate for topical use (Figure 1).

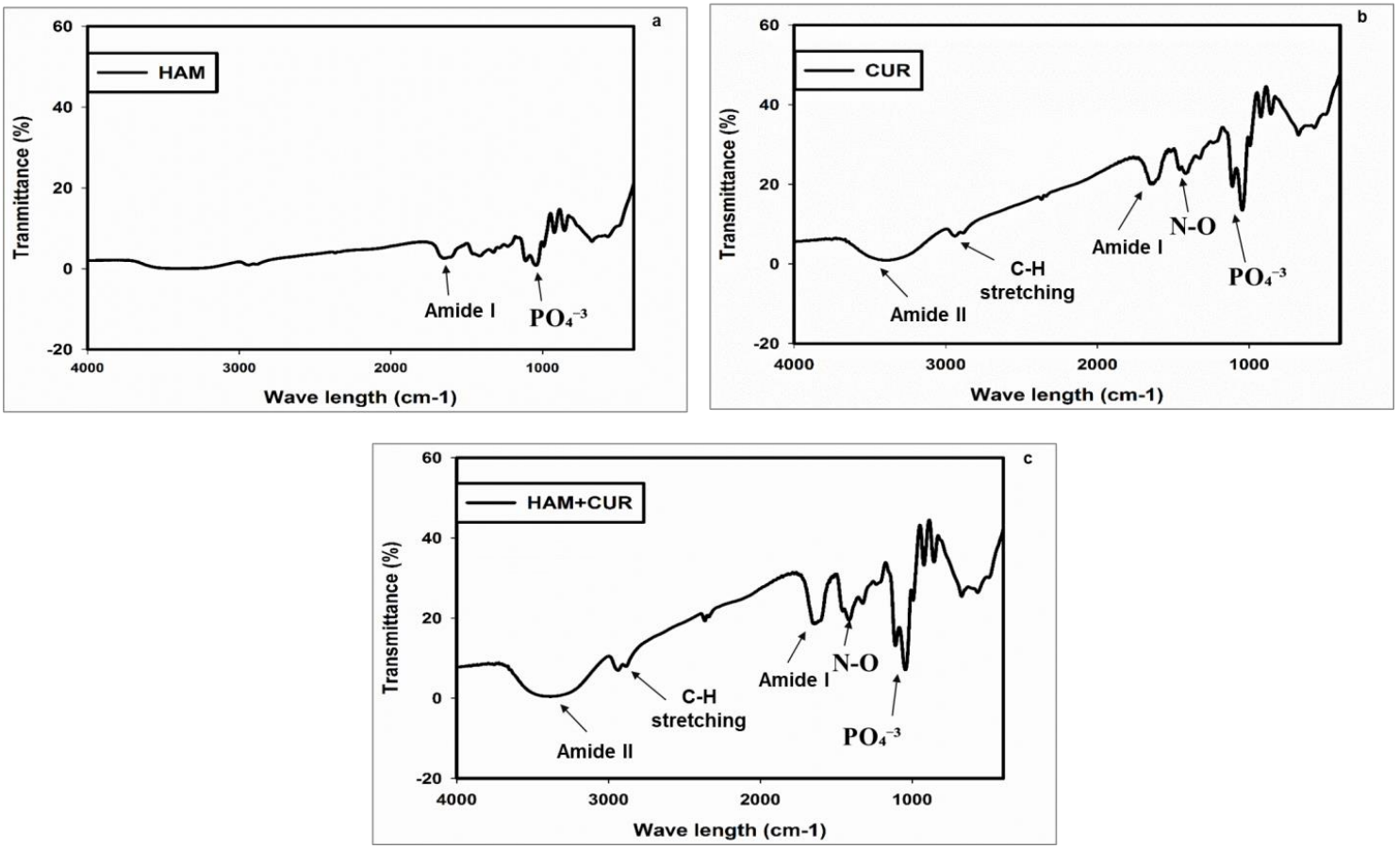

Figure 2. FTIR of the formulated gels. FTIR spectra of (a) HAM, (b) CUR, and (c) HAM+CUR

FTIR spectra of HAM, CUR, and HAM+CUR are displayed in Fig. 2. The amide-II protein absorption band in the CUR and HAM+CUR gels are shown by the peaks between 3200 and 3600 cms^{-1}, which are linked to the N–H stretching mode. Peaks in the range of (2800–3000) cms^{-1} are most likely connected to the C–H stretching mode in CUR & HAM. Moreover, the C=O stretching mode is primarily responsible for peaks between (1600–1800) cms^{-1}, which corresponds to the amide–I protein absorption band. Besides, the C-H, N-O stretching mode is represented by peaks between 1400 and 1550 cms^{-1}. The peaks located at around (1200–1350) cms^{-1} and (1000–1190) cms^{-1} are attributed to the phosphodiester group of lipids, glycoproteins, and nucleic acids. The amide III band results by the in-phase combination of N-H plane bending and C-N stretching, some of which are brought on by vibrations from C=O bending and C-C stretching.

Furthermore, we also characterized HAM, CUR and HAM+CUR gels by NMR spectroscopy. Fig.3 exemplifies the observations of NMR spectroscopy of HAM and HAM+CUR gels for the (5.324-8.002) ppm region of the spectra at 45ºC. The ^1H chemical shifts of both two gels are similar irrespective of the spectra. Here, we can identify the anomeric protons of 4-propoxy (N, N diethyl)-p hexylamide. Fig.3 also exemplifies the observation of NMR spectroscopy of CUR gel for the (0.887-1.739) ppm. This characteristic ^1H NMR signals of CUR shows no significant shifts in the ^1H NMR spectroscopy.

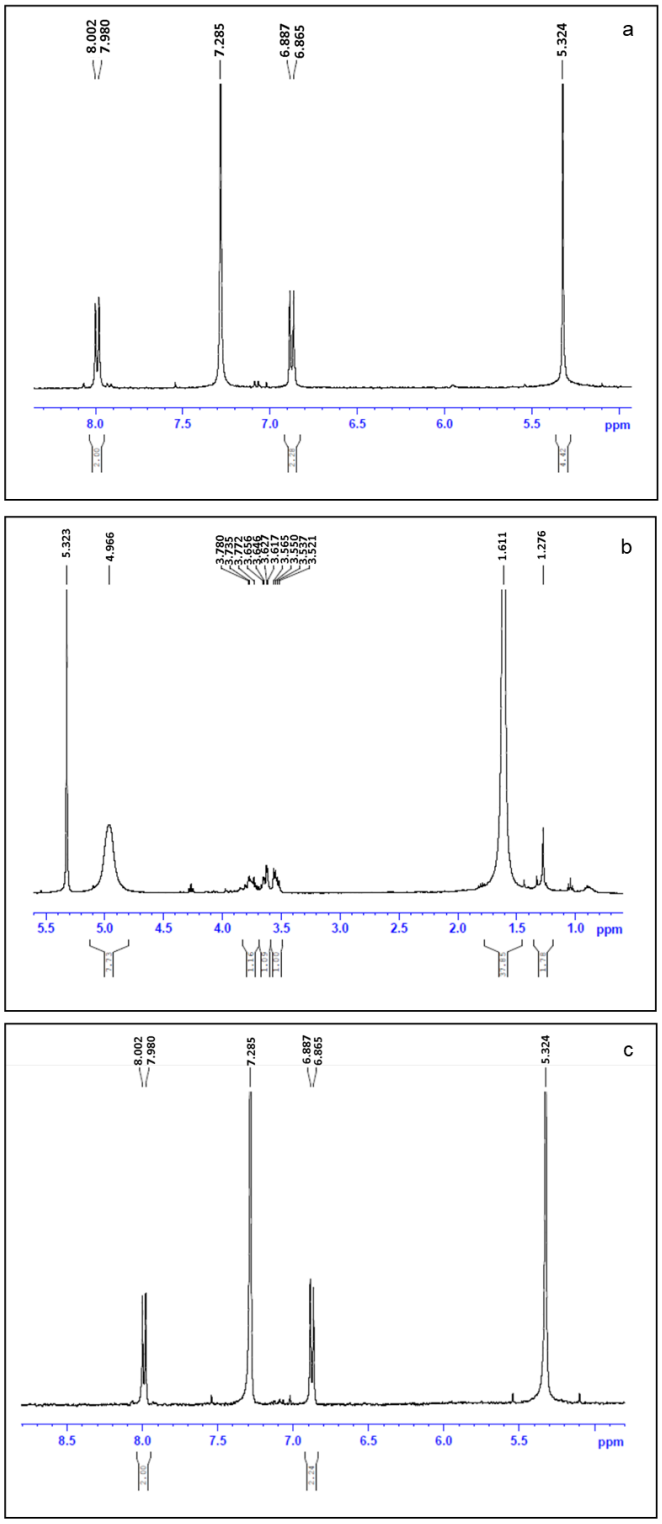

Figure 3. NMR spectroscopy of the formulated gels. (a) HAM, (b) CUR, (c) HAM+CUR

In vitro cytotoxicity test (Brine shrimp lethality test) and in vitro antimicrobial examination of the gel formulation

The results of the lethality test expressed that, in the case of higher doses of gel samples, there was an increased rate of death of nauplii and lower doses of gels showed very little or no cytotoxic effect as the death rate was minimal (Figure 4a).

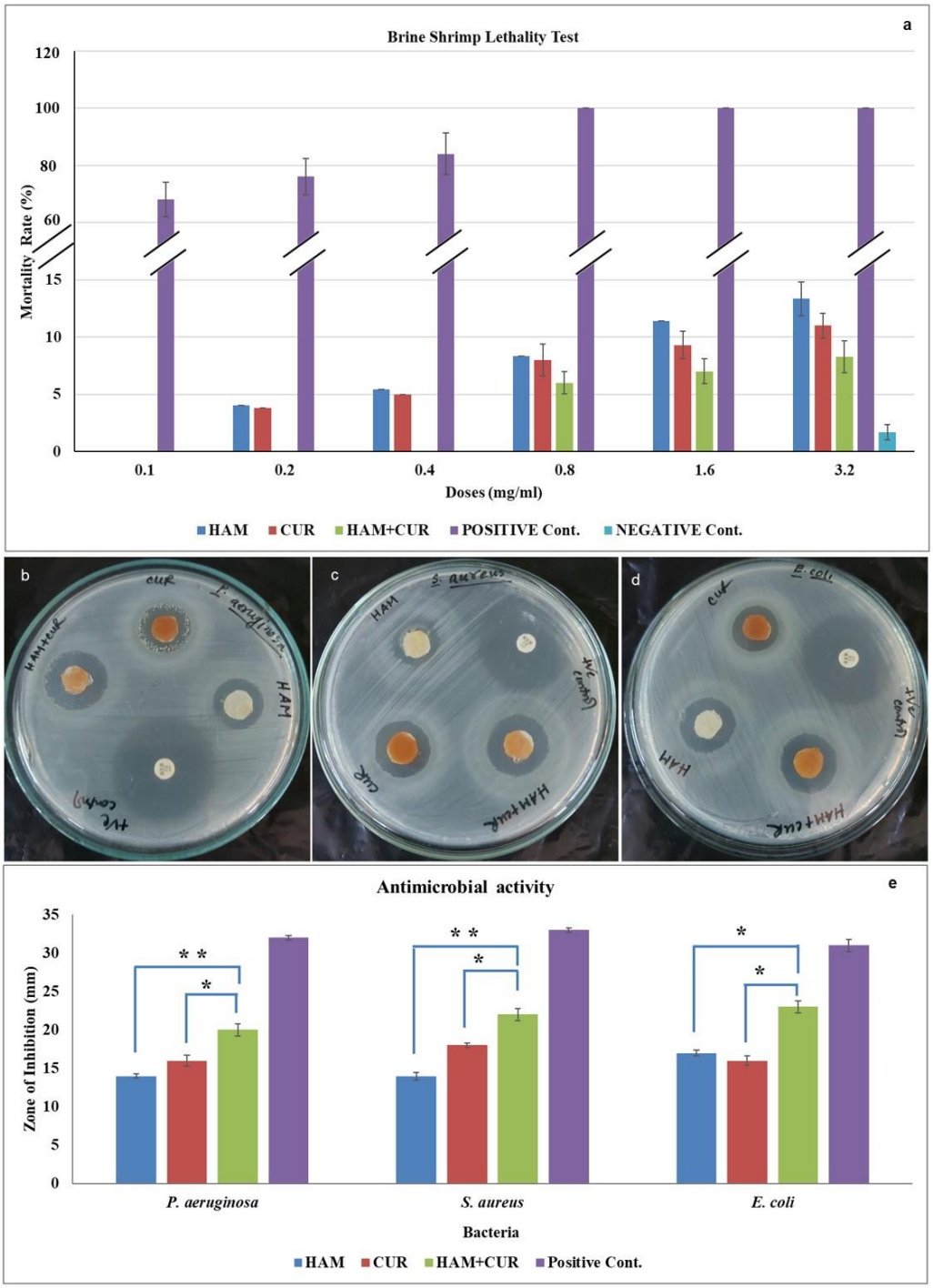

Figure 4. Brine shrimp lethality test of the gel and antimicrobial activity of the formulated gels**. (a)** Brine shrimp lethality test of the gel; mortality rate of brine shrimps at different concentrations of formulated gel. Antimicrobial activity of the HAM, CUR, HAM+CUR, and Positive Control against *Pseudomonas aeruginosa* (**b**), *Staphylococcus aureus* (**c**), *and Escherichia coli* (**d**) (level of significance was *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001). (**e**) Bar diagram of the zone of inhibition

Formulated gels expressed a little cytotoxicity at higher doses but expressed no or minimal cytotoxicity at lower doses. Compared with HAM and CUR gels, HAM + CUR gels show a larger inhibition zone against *Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli* (Fig. 4b).

Skin Irritation Study

In case of skin irritation study, it was found that the gels didn't induce any edema or erythema on the skin (Figure 5a).

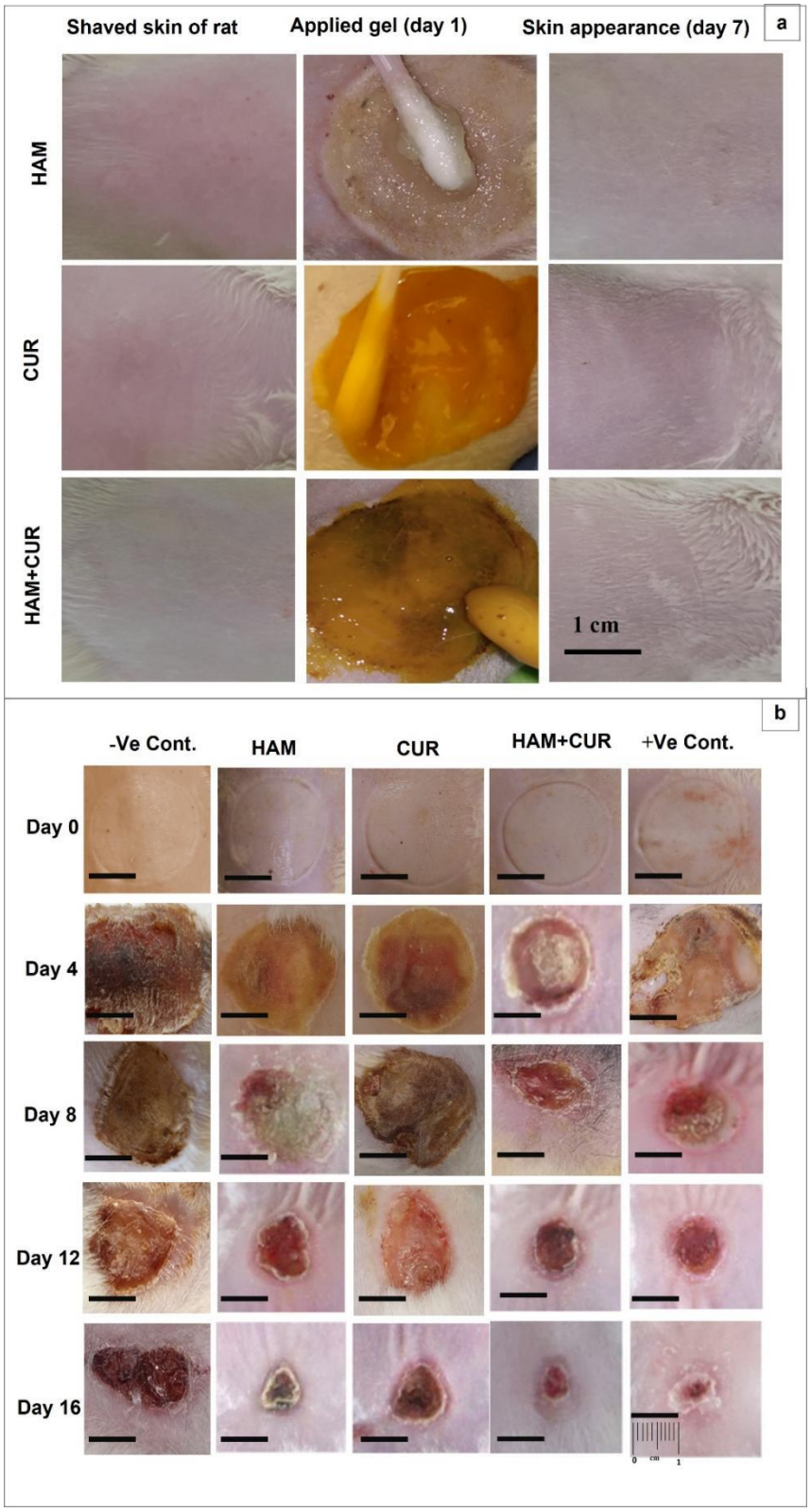

Figure 5. Skin irritation study and macroscopic observation of wound. (**a**) Skin irritation study on shaved skin: topical application of gel on day 1, and skin appearance on day 7 (no edema and erythema on the skin after 7 days), (**b)** Macroscopic observation of wound: observation of wound after applying different types of gels at day 0, 4, 8, 12 and 16

Evaluation of in vivo burn wound healing

Macroscopic observation and Percent Wound closure

The injuries and medical care for each of the time points (days 0, 4, 8, 12, 16) are illustrated in Fig. 5b. Scar tissue contraction was evident in all treatment groups on day 16. The rat groups that were exposed to HAM+CUR gel exhibited superior wound healing. By day 16, the wound area had significantly decreased. Fig. 6a displays the percent wound contraction rate.

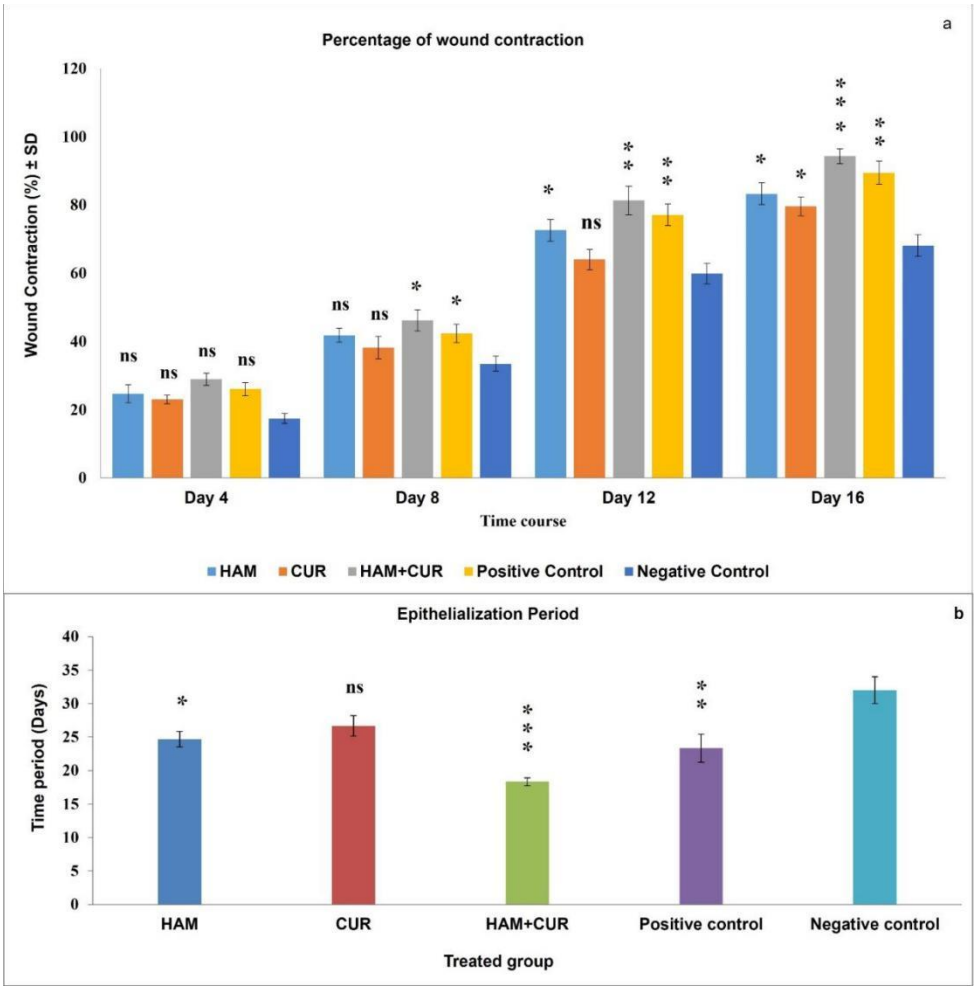

Figure 6. Wound contraction percentages and Epithelialization periods. **(a)** Wound contraction percentages of different rat groups. The graph shows that different treatment groups have significantly better wound contraction percentages compared to the negative control (*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001). Data were expressed as means ± SD. **(b)** The graph represents that different treatment groups have significantly better epithelization period compared to the negative control. Data were expressed as means ± SD. (*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001)

The percentage of wound closure in burn-wounded rats varied from group to group. The wound contraction of the negative control group was 17.43 ±1.46 on day 4, and 68.16 ±3.17% on day 16. On the other hand, the Group treated with HAM gel showed a noteworthy increase in wound contraction on day 4 (24.72 ±2.65 %), day 8 (41.82 ±2.02 %) day 12 (72.62 ±3.16 %), and day 16 (83.32 ±3.19 %), respectively. Moreover, the CUR gel-treated group showed 23.07 ±1.24 % on day 4, and 79.62 ± 2.78 % on day 16. The group treated with HAM+CUR gel burned rats showed 28.92 ±1.82 % on day 4, 46.21 ±3.01 % on day 8, 81.31 ±4.21 % on day 12 and 94.31 ±2.24 % on day 16. On day 16, the positive control groups, HAM, CUR, and HAM+CUR groups healed 89.50 ± 3.37% (P<0.01), 83.32 ± 3.19% (P<0.05), and 79.62± 2.78% (P<0.05), and 94.31 ±2.24 % (P<0.001), respectively which were statistically significant compared to the negative control. *In vivo* results indicate that treatment with HAM, CUR, and HAM + CUR gels can increase the frequency of wound healing compared to the untreated control group. The biological relevance is evidenced by the statistical observation between treatment groups.

Epithelialization Period

Periods of epithelialization in all groups of rats were also noticed and are shown in Fig. 6b. The wounds in the negative control group healed completely in 31.67 ±1.15 days, while the HAM gel-treated group needed 25.33 ±1.53 days (P<0.05), and the HAM+CUR gel-treated group required 19.67 ±1.52 days (P<0.001) for whole healing. The lower p values indicated that the epithelialization period between negative control group and HAM+CUR group had significant differences.

Histology

Fig. 7 displayed illustrations of the skin's healing process in H&E-stained form. When the burnt skin of the CUR, HAM, and positive control groups was examined histologically on day 4,

it revealed inflammatory cells and severe inflammation without granulation of tissues. However, the HAM+CUR group displayed macrophages, fibrosis, and mild granulation inflammation.

After ten days, the control, HAM, Positive, and CUR groups showed signs of significant inflammation as well as minor granulation, fibrosis, and macrophages. Conversely, on day 10, the HAM+CUR group displayed moderate fibrosis, severe granulation, mild inflammation, and macrophages. On day 16, the HAM+CUR group showed no signs of inflammation, severe tissue granulation, fibrosis, or collagen deposition, leading to wound contraction. The newly produced epidermal layer portion of the wounds treated with HAM+ CUR thickened, the scab portion reduced, the cell density increased, and the extracellular matrix (ECM) resembled normal skin. Additionally, the entire epidermis formed, and the hypodermis expanded to become more numerous. Not only the HAM+ CUR and positive control groups exhibit neogenetic papillary layers, but also neogenetic hair follicles and blood vessels. In terms of histological evaluation of wound healing, the application of HAM+CUR gel following burn injury produced the best results.

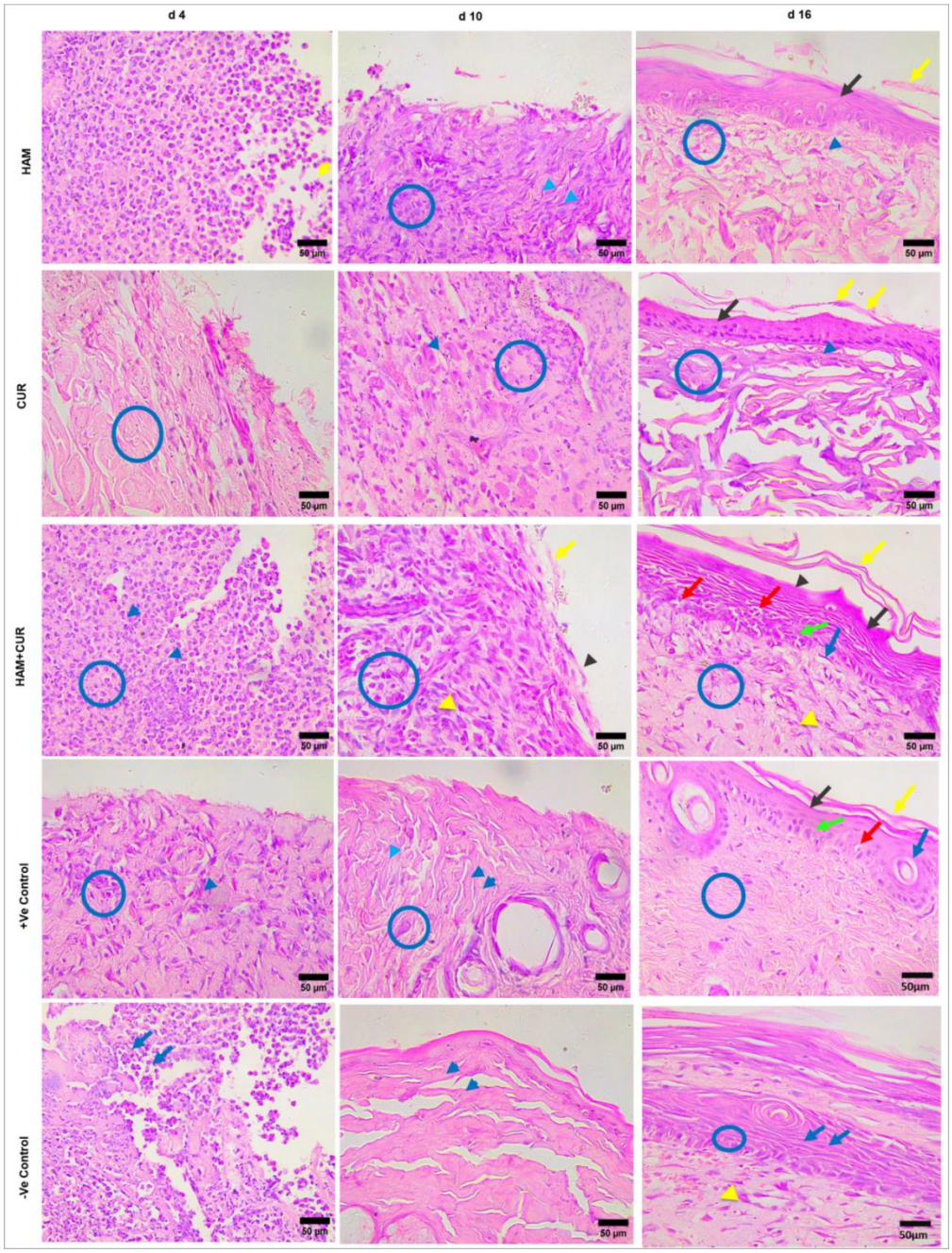

Figure 7. H&E staining. H&E staining of skin treated with HAM, CUR, HAM+CUR, Positive control, Negative control. Blue arrow, black arrow and red arrow project hair follicles, epithelial layer, and blood vessels, respectively. Collagen fibers are denoted by blue circle. Yellow arrow and blue arrow-head, point to scab part and inflammatory cells, respectively. yellow arrow-head, and green arrow express fibroblasts cells and keratinocytes, respectively (40X objective lens)

Discussion

This study evaluated the combined effects of HAM and CUR and preclinically compared their potential as a biological dressing for burn treatment to the individual effects of HAM and CUR for healing. HAM showed healing capabilities on burn wounds, which had been used in burn treatment as an efficient biological dressing material for decades (Eskandarlou et al., 2016). HAM is enriched with growth factors, anti-inflammatory compounds, and cytokines, all of which are critical for wound healing as they inhibit inflammation and the development of scar tissue (Tehrani et al., 2017; Meller et al., 2002). According to Mehrabani et al., 2015, CUR's non-toxic nature, safety for humans, and previous preclinical wound healing investigations have drawn the attention of researchers. Many investigations have been carried out to ascertain HAM's antibacterial and wound-healing properties (Yadav et al., 2017; Talmi et al., 1991). HAM's antibacterial effect is mostly attributed to the amniotic membrane peptide genes that it produces (Sangwan et al., 2011; Mao et al., 2017). CUR also prevents the growth of bacteria by rupturing the bacterial membrane's integrity (Teow et al., 2016; Li et al., 2018). Our findings extended existing evidence by showing that integrating a bioactive HAM scaffold with a phytochemical possessing CUR yields broader therapeutic effects than those reported for either material alone.

The formulated gels exhibited antibacterial properties against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli* (Fig. 4b). HAM+CUR gel exhibited a significantly greater zone of inhibition against *S. aureus* and *P. aeruginosa* compared to HAM ($P \leq 0.01$) and CUR ($P \leq 0.05$) which may be the synergistic effect of HAM and CUR (Fig. 4b).

CUR is a specialized molecule that targets bacteria in two clever ways. First, it slips into the bacteria's protective outer layer (its cell membrane), much like a key fitting into a lock, and disrupts it from the inside. Second, it triggers a kind of internal stress reaction within the bacteria, overwhelming it. HAM, on the other hand, acts like a sophisticated healing scaffold. It's naturally resistant to bacteria because it's built with built-in defenses like antimicrobial peptides and enzymes, which constantly work to suppress microbial growth. Together, they are powerful team. HAM creates a protective, pro-healing environment imagine it as a secure "operating theater." Within this safe zone, CUR can deliver its precise and powerful "surgical strike" against the infection. This synergy allows for rapid control of the infection while actively promoting the functional healing of the tissue, which is the ultimate goal (Melander et al., 2020; Memarzia et al., 2021; Dai et al., 2022). The gels in this study had pH values between 6.68 and 6.80, which is within the normal range of skin pH (Ma et al., 2010). This ensured that the formulations were suitable for the skin and would not cause irritation when applied. After applying each gel topically for seven days, none of them induced erythema or edema, suggesting that topically applying gels is safe (Fig 5a). The process of regenerating and closing the wound surface relies substantially on the wound contraction rate and the re-epithelialization profile (Suryamathi et al., 2022). HAM+CUR group outperformed other groups in the burn wound healing study, showing the quickest rate of wound healing at day 16 (Fig. 5b and 6a) and encouraging the contraction of 94.31± 2.24% of wounds (490.9 mm^2) which was highly significant ($P \leq 0.001$). The highest rate of burn wound healing was found in the HAM+CUR group in this investigation, implying a biologically meaningful improvement. Another study demonstrated that on the 24th day, rats treated with HAM and gel produced from *Moringa oleifera* leaves displayed 96 ± 1.96% wound contraction (Islam et al., 2018). Using an albino rat model, Mehrabani et al., (2015) previously showed that 2% curcumin treatment reduced the size of the burn wounds and the level of inflammation after 14 days. In a study, only HAM based gel showed wound contraction of about 70±1.25 % where as in this study the combined gel showed 94.31 ±2.24 % on day 16 (Akter et al., 2025). According to earlier research by Adeliana et al., (2021), rabbits' wounds heal more quickly, edema is reduced, and epithelium development is accelerated when 5% CUR gel is applied. Here, the combination of both HAM and CUR synergistically improved epithelialization. The combination HAM and CUR have not been observed before in burn wound healing experiment which has been addressed in this study.

For second-degree burns, it typically takes 25–35 days for the wound to fully heal (Ramli et al., 2011). In this investigation, the burn lesions treated with HAM + CUR gel healed completely ($P<0.001$) in just 19.67 ± 1.52 days, reaching the maximal degree of epithelialization (Fig. 6b). A comparable study found that the epithelialization period for HAM gel containing silver nanoparticles was 23.67 ± 2.05 days (Jhumi et al., 2023). In histological observation of H&E-stained tissues, the HAM+CUR group enhanced the regeneration of the epidermal layer and boosted granulation tissues, collagen fibers deposition, sebaceous glands, and skin appendages like hair follicles (Fig. 7). Hair follicle formation and epidermal regeneration are crucial for skin wound healing and re-epithelialization (Somboonwong et al. 2012). The phases of collagen synthesis, deposition, and maturation are also essential for tissue regeneration and skin rebuilding following wound closure (Jhumi et al., 2023). Comparing HAM+CUR gel to control and other groups, all these results indicate that the gel promoted wound skin renewal via epithelialization, angiogenesis, collagen synthesis, and scar formation.

There are some limitations in this study, including molecular pathways (e.g., cytokine profiling, gene expression, signaling cascades, fibroblast activity) underlying the synergistic effects of HAM and CUR were not examined, no inflammatory or oxidative biomarkers were quantified; thus, the biological basis of the wound-healing effects remains indirect. Besides long-term assessment of scar quality and delayed inflammatory reactions were not performed. Further studies incorporating mechanistic analyses, dose–response evaluations, safety testing, and larger sample sizes, as well as preclinical and clinical benchmarking are required to validate HAM+CUR as a therapeutic candidate. These limitations will be addressed in our future study.

Conclusion

We successfully synthesized HAM+CUR therapeutic gel by combining HAM and CUR powder for treating burn wounds. Application of this gel resulted faster burn wound healing in rats associated with the reduction of the epithelialization period and enhancement of the wound contraction rate. Edema and

erythema were non-existent after application. These findings were also supported by histopathological analysis which revealed the formation of new epidermis, angiogenesis, collagen formation, and the formation of granulation tissue. Therefore, HAM + CUR gel can be used as an effective wound healing therapeutic agent for treating burn wounds. However, more research is needed to determine the molecular mechanisms of action of wound healing.

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Disclosure
Hafizur Rahman, Ayesha Siddika, Polash Chandra Karmakar, Tusher- Al-Arafat are the co-first authors.

Compliance with ethical standards
The collection and use of cesarean sections derived amniotic membrane for research and grafting purpose was approved by the "Human Organ / Tissue Donation and Transplantation Act, 1999" Govt. of Bangladesh. Written consent from the amniotic membrane donor was taken for amniotic membrane collection for use in research purpose.The 'Biosafety, Biosecurity and Ethical Committee' of Jahangirnagar University, Savar, Dhaka, Bangladesh has provided the ethical permission [Ref no: BBEC, JU/M 2020 (11) 3] following ARRIVE guidelines. All efforts were made to prevent any unnecessary and harmful animal handling.

Conflict of interest
The authors declare that there is no conflict of interest.

Authors contribution
All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Hafizur Rahman, and Ayesha Siddika, Liakat Hossain, Hasib Adnan performed skin irritation studies. Polash Chandra Karmakar and Tusher- Al-Arafat performed histological analysis. Ayesha Siddika, Polash Chandra Karmakar, and Tusher- Al-Arafat wrote the manuscript. S.M. Asaduzzaman, Naznin Akhtar, and Farha Matin Juliana edited the manuscript. All authors read and approved the final manuscript.

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