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ORIGINAL PAPER

Wound healing potential of *Apamarga Ksharodaka* (herbal alkaline water made from *Achyranthus aspera* Linn.) in excision rodent wound model

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ABSTRACT

Introduction and aim. Wound healing is a biological process that aims to restore tissue integrity and function. Despite medical advances, wound management remains challenging. Traditional medicinal preparations, like *Apamarga ksharodaka* (AK), offer promising therapeutic potential due to their phytochemical richness. This study evaluated wound healing and antimicrobial activity of AK. This study aimed to validate the traditional claim of AK's wound healing potential using an excision wound model.

Material and methods. An excision wound model was created using 24 male Wistar rats. A positive control group applied 5% w/w povidone-iodine (PI) ointment. Wound contraction (WC), epithelialization period (ET), wound closure day, and histopathology were assessed. Antibacterial activity was evaluated against *Escherichia coli*, *Streptococcus aureus*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*.

Results. AK showed slightly better wound healing than PI ointment, with significant results in WC rate, wound closure, and ET. Histopathology revealed normal skin and organ architecture. The minimum MIC was 6.25 mg/ml against *Pseudomonas aeruginosa with* a maximum inhibition zone of 15 mm.

Conclusion. AK is safe and effective for wound healing.

Keywords. antimicrobial activity, *Apamarga ksharodaka*, excision wound model, phytochemical richness, traditional medicinal plants, wound, wound healing

Introduction

Disruption of normal anatomical structure and function of the skin is called a "wound".[1] Healing is a series of events typically involving hemostasis, inflammation, proliferation, and remodeling with scar formation phases.[1] Clinical management strategies for wounds are aimed at preventing infection and accelerating healing. The management of wounds with a sterile dressing, the administration of antimicrobial, anti-inflammatory, analgesic drugs and promoters of wound healing augments the healing of wounds.[2]

Achyranthus aspera Linn. commonly known as *Apamarga* in *Ayurveda*, has been described as a divine medicine. *A. aspera* has rich medicinal values and is used as an important ingredient in various formulations. It has been reported to possess activities such as antihyperlipidemic, wound healing, hepatoprotective, antimicrobial, diuretic, antioxidant, anti-inflammatory lithotriptic,

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etc.[3] It is described in the Ayurvedic literature as an expectorant, carminative, digestive and blood-purifier.[4]

Kshara (herbal alkalizer in dry and powdered form) is considered a parasurgical instrument in Ayurveda. Healing and purification of wounds are special properties of *Kshara*.[5] External application of *Kshara* is indicated in many diseases such as skin disorders, nonhealing ulcers, poisoning, psoriasis, piles, fungal infections, worms, sinuses, mouth disorders, tumors, fistula, etc.[6] An intermediary aqueous preparation of *Kshara* is known as *Ksharodaka* (alkaline water). In Ayurved classics, the external application of *Ksharodaka* is recommended for various diseases, that is, in the treatment of goitre, and dandruff.[7] In the context of parasurgical procedures, *Ksharodaka* is mentioned for washing the wound for deworming.[8] *Apamarga kshara* is widely used to treat nonhealing ulcers and anorectal diseases. *A. aspera* Linn is the most commonly used plant for the preparation of *Kshara*. The ease of availability and its pharmacological characteristics are favorable for wound healing (antimicrobial, analgesic, anti-inflammatory, hemostatic, etc.) and exemplify an ideal agent for wound healing.

Aim

For the treatment of fresh excised wounds, *Apamarga Ksharodaka* (AK) is not commonly used and is also not mentioned in the classical ayurvedic literature, so in the present study, AK was selected to validate the traditional claim and attempts were made to evaluate the healing potential of AK in the excision wound model.

Material and method

Animals

Wistar Albino male rats, weighing 200–250 g were selected for the present study. The animals were received from the IMS animal house, Banaras Hindu University (BHU), Varanasi. Rats were kept in the Animal House of the Centre of Experimental Medicine and Surgery, IMS, BHU at 26±2°C and relative humidity of 44–56%, with light and dark cycles of 10 and 14 hours, respectively, for 1 week before and during the experiments. under standard conditions of temperature (22±2°C), humidity and 12-hour light /dark cycle, and relative humidity of approximately 50–55% with a normal diet and purified water *ad libitum*. The animals were acclimatized to standard experimental conditions for 7 days prior to initiation of the experimental study. The CPCSEA guidelines were strictly followed throughout the study. Before the experimental work, approval was obtained from the Institutional Animal Ethical Committee (IAEC) of IMS, BHU (Dean/2010-11/173 dated 23/07/2010). The experiments were carried out during the months from November to January.

Preparation of herbal formulation

The fresh mature *Apamarga panchanga* (all parts of *A. aspera* plant) was collected from the local supplier of Varanasi and weighed. Authentication was performed at the Department of Botany, IMS, BHU. The voucher sample has been kept in the herbarium. (Voucher specimen No. Amarantha 2023/03). *Apamarga Ksharodaka* (AK) was prepared using the classical method of *Ayurveda*.[9] The plant was washed with water to remove physical impurities and then placed for drying in sunlight. When it was completely dried, it was weighed again on the digital weighing machines. The dried plant was placed in a large earthen pot and completely burnt and grey ash was collected after self-cooling, free from stones, mud, and charcoal. The ash was collected in a glass vessel and 6 times of water was added to it, then the contents and left undisturbed for the next 24 hours. The liquid layers of the clear supernatant were collected via an outlet and filtered 21 times filtered using a 3-layer cotton cloth. This *Ksharodaka* was heated over a laboratory hot plate at 60° fixed temperature with intermittent stirring until it remained 1/3 of the total and turned sticky, transparent, and red.

Excision wound healing activity

Male Wistar rats with good health condition, weighing between 200–250 g were randomly divided into four groups (6 rats per group). Group I was a negative control group and did not receive any intervention, and group II was the positive control group that applied 5% pomidine iodine (PI) ointment. Group III was test group 1 received the test drug AK followed by 5% w/w povidone iodine ointment (PI) and Group IV was test group 2 applied AK only.

Excision wound model

The partial-thickness excision wound model was used as described by Morton and Malone.[11] Rats were subjected to ketamine anesthesia (IP) and the skin of the rats was shaved in the mid-scapular region and full-thickness skin wounds were established with a diameter of 2 cm diameter and 0.2 cm depth of punches. These wounds were generated by surgical removal of all skin layers (epidermis, dermis, and subcutaneous fat).

After full recovery from anesthesia, the animals were individually housed in cages. Topical administration of all drugs was conducted daily on wounds till they healed. Wound contraction was measured by planimetric measurement of the wound area. The duration of complete epithelization was determined by noting the days until the scab fell off and no raw wound behind. The percentage of wound contraction and epithelization period was also accessed for healing.

Method of application of test drug

The surrounding area of the wound was covered with a gauze piece to prevent the spread of the drug on healthy tissue. The test drug (AK) was dropped on the other gauze piece with the help of a dropper. Approximately 450 mg of gauze absorbed the test drug (AK) with complete soackage achieved by 10 drops (0.5 mL). AK was applied to the proposed lesion by probing up to the count of 100. As soon as the sign of proper cauterization appears (as described by Sushruta).6 AK is rapidly neutralized by dropping acidic fluid (lemon water) on the wound to neutralize the drug. The wound was washed out with distilled water and the dressing was done. The wound was monitored using signs from the relevant article, that is, after applying AK, the tissue of the treated area becomes purple or dark black, the cauterized lesion shrinks and pain and discharge are relieved.[10] Any adverse effect during the application of AK such as redness of the cauterized area, ulceration with purulent discharge, fever, severe pain, shock, etc. was carefully monitored to evaluate the safety of *Ksharodaka*.

Assessment of wound contraction

Wound contraction was evaluated by monitoring changes in the wound area using planimetric techniques, excluding the day of wounding. The sizes were traced onto transparent paper at specific intervals of 4 days (ie, on the zero, 4^{th}, 8^{th}, and 12^{th} days) and then on alternate days until complete wound healing occurred. These traces were noted onto a 1 mm^2 graph sheet to quantify the wound surface area. Using the following formula, the percentage of wound contraction was calculated.[11]

$$\text{\% Wound contraction} = \frac{\text{(Initial wound size - wound size on a specific day)}}{\text{Initial wound size}} \times 100$$

The duration needed for Escher to fall away without leaving a raw wound was used to monitor epithelialization time (ET).[12] The day the raw surface reaches the level of the skin and develops a scab on its upper surface is recognized as wound closure day.[13] To evaluate the quality of wound healing, an excisional biopsy of the healed skin was analyzed for histological evaluation.

Histopathology

After complete epithelization, wound tissues were excised and stored for 24 hours in 10% buffered formalin. Subsequently, histopathological analysis of regenerated skin tissue was performed following the same procedure as in liver, brain, heart, and kidney histopathological analysis.

In vitro antimicrobial activity

The antibacterial activity of AK was tested using the disc diffusion technique.[14] Muller-Hinton Agar (MHA) plates were developed by placing 15 ml of the molten medium into sterile Petri plates. The recently cultured bacteria were suspended in sterile saline to obtain 107 colony-forming units (CFU) per ml concentration. The suspension was then spread evenly on the surface of Mueller-Hinton agar (MHA) plates and allowed to air dry for 5 minutes. Then 5 µl drug in 300 mg/ml concentration was sited on 6-mm sterile discs of Whatman filter paper no.1. Subsequently, these discs were placed onto the agar surface to allow the compound to diffuse for 5 minutes. The plates were then incubated under specific conditions of 37°C for the 24 hours for cultures. After incubation, the zone of inhibition (ZOI) was measured near the discs with the help of a ruler.

Statistical analysis

Data were presented as mean ± standard error (SE). Statistical significance between the control and treated groups was evaluated using analysis of variance (ANOVA), followed by the student's t-test and post hoc test. A significance level of $p<0.05$ was considered significant. All statistical analyzes were performed using GraphPad Prism software (GraphPad Software, Boston, MA, USA).

Results

Excised wound healing activity

The progression of wound healing in all groups is shown in Figures 1 and 2. The wound contraction (WC) in control group rats was 12.22% to 87.78% from day 4 to day 16, in test group 1 it was 84.44% on day 16. Complete skin healing was observed on day 19 in both these groups. In the positive control group, wc was 96.67 % on day 16 and 95.54 % in test group 2, and skin normalization was observed on day 17 in both these groups (Table 1, Figures 1 and 3).

Table 1. Comparison of all groups for the percentage of WC at different time points

		% of wound contraction					Mean area of wound (mm^2)				
Groups (n=6 in each)	Summary	Day 4	Day 8	Day 12	Day 16	Day 21	Day 4	Day 8	Day 12	Day 16	Day 21
Negative control	Mean±S.E.	12.22± 2.68	45.56± 3.18	65.56± 3.62	87.78± 2.05	97.78± 1.41	547.38± 10.45	213.19± 8.06	88.52± 5.46	12.05± 1.19	1.05±0.21
Positive control	Mean±S.E.	8.89± 3.30	25.56± 5.82	73.33± 4.55	96.67± 2.28		590.86± 13.49	403.86± 12.80	57.62± 5.80	2.62± 0.65	
Test group 1	Mean±S.E.	32.28± 5.27	52.50± 5.92	66.11± 5.46	84.44± 3.14	94.44± 2.08	459.73± 18.87	136.71± 12.77	56.05± 9.96	13.62± 2.74	2.62±0.85
Test group 2	Mean±S.E.	17.51± 1.91	39.04± 2.71	70.10± 2.61	95.54± 2.85		632.55± 14.24	396.52± 9.54	100.05± 6.94	6.81± 1.51	

Epithelialization time

The positive control group (PI) (mean=12.83) showed a significant reduction (p=0.0418) in ET compared to the control (mean=14.83) and test Group 2 (AK) (mean=11.83) also showed a significant reduction (p=0.0201), while test Group 1 (AKPI) (mean=14.67) was not significant (p=0.9947) (Table 2).

Wound closure day

Significant results were observed in test group 2 (p=0.0110), positive control group (p=0.3081), and test group 2 (p=0.011), while test group 1 was not significant p=0.9735 (Table 2).

Table 2. Comparison of all groups with respect to ET and wound closure day

Groups (n=6 in each)	Summary	Epithelialization time	Wound closure day
Negative control	Mean±S.E.	14.83±0.60	12.33±0.76
Positive control	Mean±S.E.	12.83±0.48	10.67±0.21
Test group 1	Mean±S.E.	14.67±0.49	12.17±0.48
Test group 2	Mean±S.E.	11.83±0.31	9.67±0.33
p		0.0041	0.0314
F		9.024	10.69

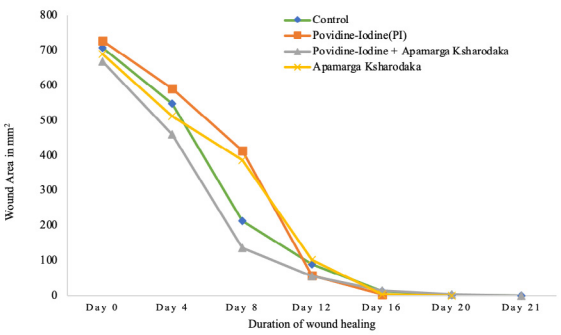

Fig. 1. Graphical presentation of wound healing activity

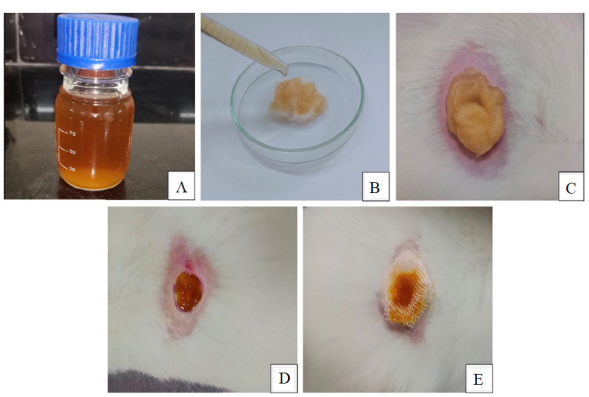

Fig. 2. A: Test drug (*Apamarga Ksharodaka*), B: *Cotton soaked with Apamarga Ksharodaka* (10 drops or 0.5 ml for complete soak), C: Wound dressing with *Apamarga Ksharodaka* in group 4, D: Dressing with 5% w w Povidone iodine ointment in group 2, E: The wound was covered with sterile cotton and gauze in group 2

Histopathology of healed skin and organs

Histopathological observations of the skin section for all groups showed normal blood vessels, dermal and epidermal layers. Histology of liver sections in all groups showed no histological abnormalities with visible hepatic lobule/lumen and well-preserved liver cells in hepatocellular architecture. All groups showed normal histology of the rat cardiac muscles. The brain sections in all groups showed no histological abnormalities and normal blood vessels and pyramidal cells. Histopathological observations for all groups showed normal histology of the rat kidneys (renal tubules, renal cells) was found (Fig. 4).

Antimicrobial analysis

Table 3. *In vitro* MIC-MBC value of AK against different bacteria (in mg/ml)*

Organism	MIC	MBC	ZOI
Escherichia coli	12.5	25	10 mm
Streptococcus aureus	12.5	25	10 mm
Pseudomonas aeruginosa	6.25	12.5	15 mm
Acinetobacter baumannii	25	50	6 mm

* MIC minimum inhibitory concentration, MBC minimum bactericidal concentration, ZOI zone of inhibition

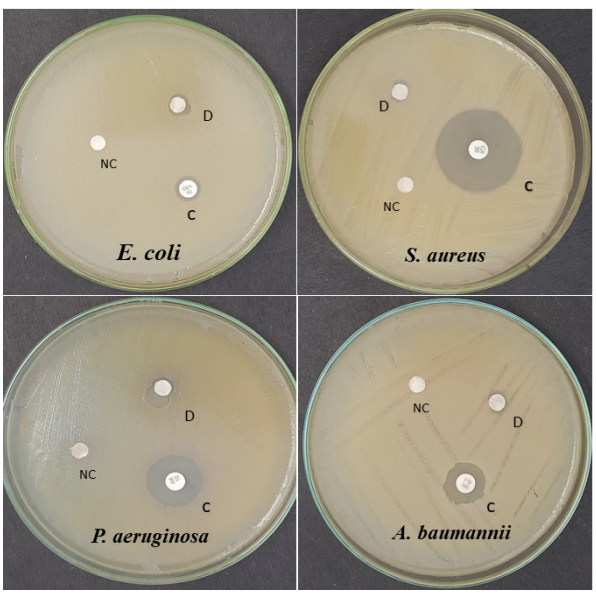

Fig. 5. Antimicrobial activity of AK by the Agar-plate diffusion method

For different bacteria, AK (5 µL drug at a 300 mg/mL concentration) showed the minimum MIC value (6.25 mg/mL) against the *P. aeruginosa* (Gram-negative) bacteria (negative Gram) compared to the other Gram-negative and positive bacteria. The maximum ZOI (15 mm) was expressed against *P. aeruginosa* (Table 3, Fig. 5).

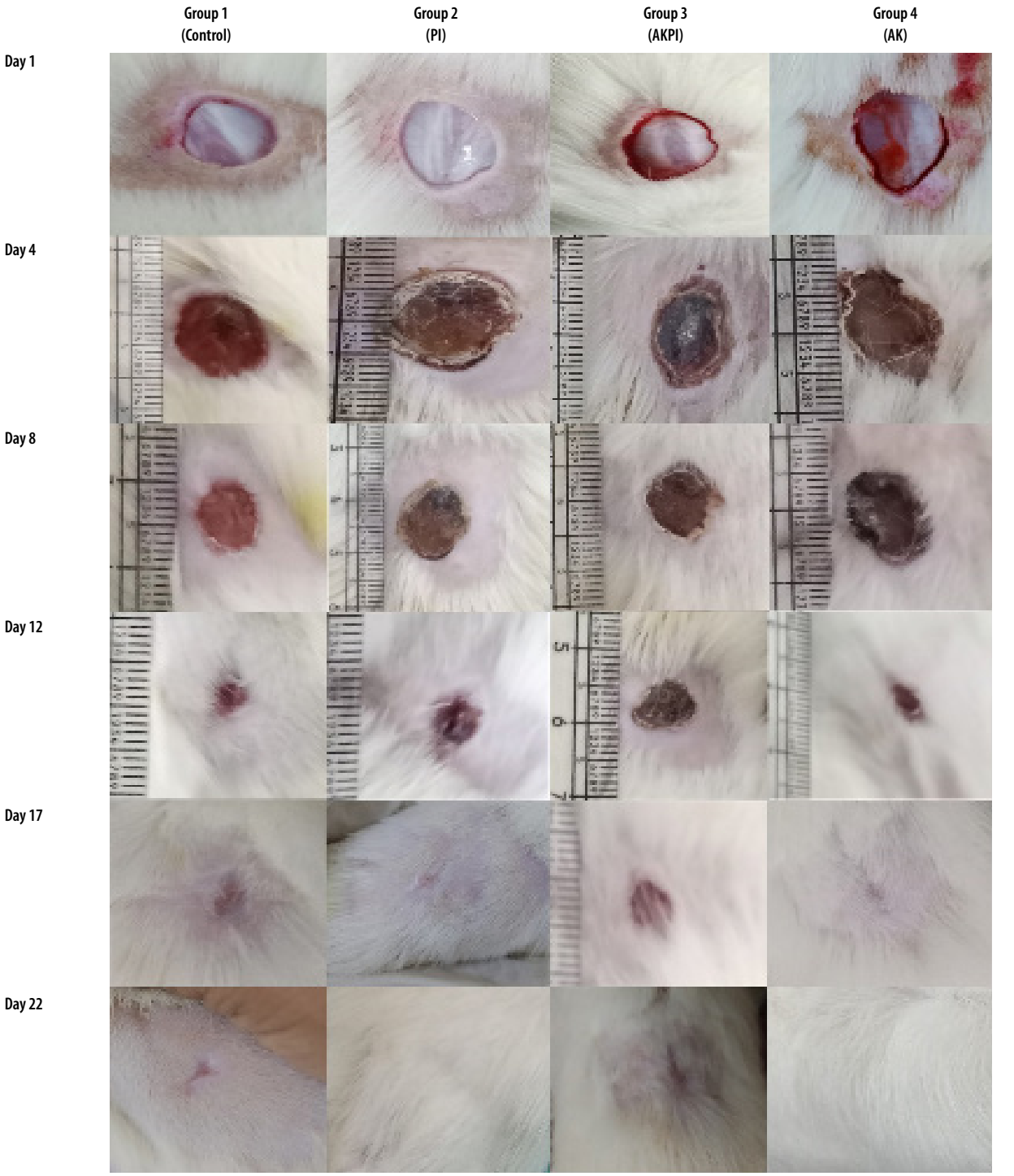

Fig. 3. Photographic representation of WC on different days (group 1 was negative control who received no intervention, group 2 was positive control who received PI ointment 5% w/w, group 3 was test group 1 who received AK and PIointment 5% w/w, and group 4 was test group 2 treated with AK)

Discussion

Wound management aims to enhance tissue regeneration and prevent associated risk factors like infection that directly influence the healing process. Wound healing progresses through distinct phases composed of complex cellular and molecular events. Initially, hemostasis occurs to stop bleeding, followed by an inflammatory phase in which immune cells clear debris and pathogens. Subsequently, proliferative processes promote tissue rebuilding, with fibroblasts producing collagen and new blood vessels forming. Finally, remodeling refines the structure and strength over time, completing the dynamic wound repair process.[1]

AK is an herbal formulation that is not commonly used for a fresh excised or incision wound. An acute dermal toxicity study was also performed to ensure its safety before starting the experiment in animals.

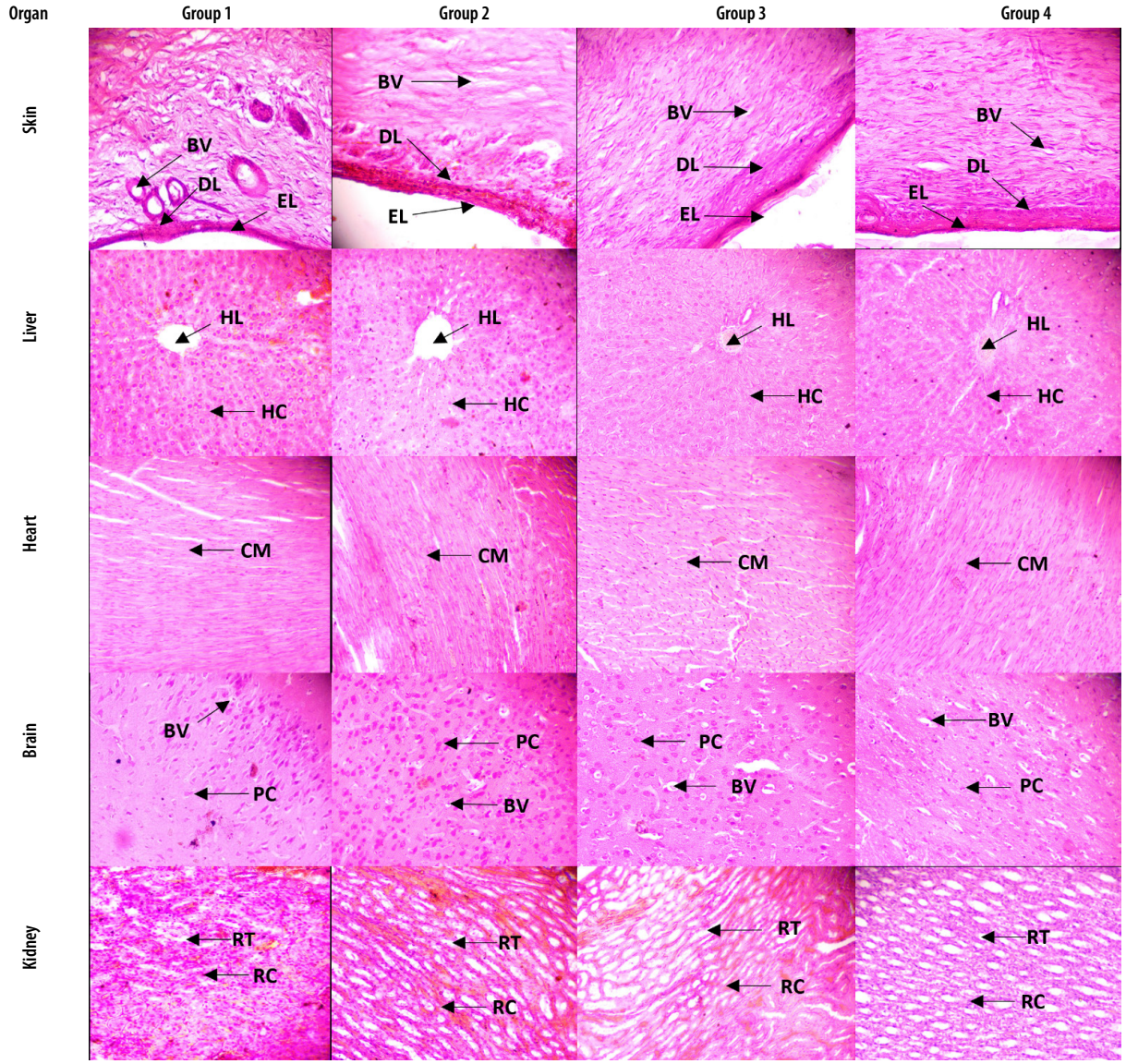

Fig. 4. Tissue sections of different organs and their histopathology on the 21st day after treatment (BV blood vessels, DL – dermal layer DL, epidermal layer, HL – hepatic lobule/lumen, CM – cardiac muscle, HC hepatocyte, PC – pyramidal cells PC, renal tubules, RC – renal cells RC)

WC refers to the process in which the size of the wound decreases due to the pulling together of the wound edges by specialized cells called myofibroblasts. Myofibroblasts are a type of fibroblast that contain contractile proteins, allowing them to exert forces and pull on the surrounding tissue. This cell contraction contributes significantly to reducing the overall size of the wound during the healing process, ultimately aiding in wound closure and scar formation.[15]

The results of this study demonstrated a significant effect of AK on promoting wound contraction, reducing epithelialization time, and a potent antimicrobial action. The results of wound contraction clearly showed differences in the rate and extent of healing between the groups. In the present study, the test drug (AK) and PI showed a significant WC rate ($p<0.0011$) (mean difference (MD)=2.667)) for AK and ($p<0.0045$) (MD=2.550) for PI itself compared to the control ($p<0.3080$) (MD=1.545). Wound healing consists of an orderly progression of a series of stages that establish the integrity of damaged tissues. Wound infections represent the invasion of tissues by the number of species of microorganisms. *A. aspera* has antibacterial and anti-inflammatory properties, and researchers have also claimed that it to be good for skin diseases and also a wound healer, cleanser and removes body toxins, etc.[16] The phytochemicals present in AK play a role in preventing the extension of the initial phase of wound healing by reducing infection, thus facilitating the process of wound contraction. The significant result of wound contraction for AK shows the healing potential through the topical route.

The drug *A. aspera* is delivered in the form of *Ksharodaka* (alkaline herbal water), which is highly alkaline (pH=12.75). High pH drugs create an environment that

supports the activity of certain enzymes involved in tissue repair, so they can contribute to wound healing. This alkaline environment helps deactivate harmful bacteria and enzymes that can delay healing. High pH solutions may help soften necrotic tissue and promote wound debridement. However, its use requires careful monitoring to avoid potential adverse effects on healthy tissue surrounding the wound.[17]

PI (mean=12.83, p=0.0418) and AK (mean=11.83, p=0.0201) showed a significant reduction in epithelialization time compared to control. In between all groups, AK showed the fastest epithelialization. Reduction in epithelialization period attributed to enhanced collagen synthesis, leading to increased skin tensile strength in excision wounds.[18]

Significant results were observed in AK (mean=9.87, p=0.0110) on wound closure day compared to control. Between all groups, AK showed the minimum wound closing time. Hemostasis involves platelet aggregation and clot formation, followed by inflammation with immune cell infiltration. During proliferation, fibroblasts synthesize extracellular matrix, leading to tissue reconstruction, and remodeling involves collagen reorganization for wound maturation.[19] Enhancement of collagen synthesis by fibroblasts, stimulation of neoangiogenesis, and increase of granulation tissue formation are attributed to the presence of phytochemicals in *A. aspera* (alkaloids, flavonoids, saponins, and tannins), which accelerates the process of tissue regeneration.

Bacterial skin infections commonly lead to other systemic diseases by disrupting the immune system and inducing inflammation, tissue impairment, and resulting in delayed wound healing. An antimicrobial study was performed to evaluate the antibacterial activity of AK. This pharmaceutical preparation can be used to treat topical infections of *P. aeruginosa*, *A.baumannii, S. aureus,* and *E. coli.* The MIC and MBC values, particularly against *P. aeruginosa,* are significant. AK was more effective against *P. aeruginosa* (MIC 6.25 mg/mL, MBC 12.5 mg/mL) with a zone of inhibition (ZOI) of 15 mm, followed by *E. coli* with a ZOI of 10 mm. This indicates the potential of AK to target both Gram-negative and Gram-positive bacteria, supporting its use in wound healing, where infections from these pathogens are common. The antimicrobial action of AK may have contributed to faster wound healing by preventing bacterial colonization and subsequent infection. Previous studies show that *A. aspera* possesses an antibacterial potential, which is confirmed in the current study by inhibition of bacterial growth. Sofowora reported that secondary metabolites (such as alkaloid, phenol, flavonoid, steroid, saponin, glycoside, oil, and tannin) in plant extract are traditionally used in the treatment of infectious wounds and have potential antimicrobial activity that justifies their traditional use for the treatment of various illnesses.[20] Yadav et al. found antibacterial activity in the aqueous extract of *A. aspera* against *Streptococcus mutans*.[21] A similar study was performed by Kaur et al., and found the antibacterial potential of chloroform and methanol shoot and root extracts against *Klebsiella sp.* and *Bacillus Substilis*.[22] Owk et al. proved the aqueous extract of plants has a maximum inhibition zone against *B. subtilis and S. aureus;* while considerable inhibitory activity against *Streptonryces pneumoniae*.[23] This inhibitory effect of extracts against human pathogens introduces this plant as a potential substance for the development of new drugs against pathogenic microbes.

Histopathological observations of the skin section for all the groups showed normal blood vessels, dermal and epidermal layers. Histopathological observations for all groups showed normal architecture of rat kidneys, liver, brain, lungs, and spleen cells. All groups did not show histological abnormalities (Fig. 4).

Many studies are carried out with various herbal medicines and plant extracts that have wound-healing properties.[24] Results indicate that the aqueous and ethanol extracts of *A. aspera* significantly promoted the tensile strength of the wound using an incision and excision wound. The aqueous extract has more superoxide scavenging and DPPH radical scavenging activity compared to the ethanolic extract.[25] A study revealed that the 80% methanol leaf extract of *A. aspera* with 10% chloroform fraction possessed a high degree of WC, a reduced period of epithelialization, and also had significant anti-inflammatory activity.[16] In this research, the wound healing activity in AK is supported by the reduction in the rate of WC, ET, histopathological evidence, potent antibacterial activity, no secondary infections, and no morbidity. This study also validates the traditional claim for AK for wound healing.

The result obtained from this study restores the facts explained by our Acharyas regarding the healing properties of *Ksharodaka.* This echoes the vast knowledge and deep insight of our Acharyas in designing *Ksharodaka* as a separate dosage form. The current observations can be considered as a basis for further studies on *Kshara Kalpana.* This study gives valuable considerable research that has focused on the validation of *Apamarga Ksharodaka* for wound healing activity.

Study limitations

The study was carried out on a relatively small number of animals (24 rats). A larger sample size could enhance the applicability of the findings, and testing on additional species may provide broader insights, especially to translate the results to human applications. The short duration of the study restricts insights into the long-term effects of AK. Variability in the traditional preparation of AK may affect the consistency of the result, so standardization of the formulation could help ensure more reliable and reproducible outcomes across different studies.

Future perspective

Future research should focus on conducting clinical trials with humans to test how safe and effective AK is for wound care and to identify any possible side effects. Comparative studies with other herbal and modern wound healing agents would provide information on their relative effectiveness. Optimizing dosage and standardizing formulation is essential for consistency, and exploring the molecular mechanisms underlying AK's effects could further clarify its therapeutic potential.

Conclusion

This study revealed that in the topical application of AK, there were no signs of dermal toxicity. The wound healing activity in AK was slightly more potent compared to PI ointment. AK showed pronounced antimicrobial and healing potential, so it may be suggested for treating various types of wounds. AK showed a significant improvement in WC rate, wound closing period, ET, and histopathological changes. This study shows that AK is a safe and effective drug for wound healing.

Declarations

Funding

The author declares that no funding was received for conducting this research.

Author contributions

Conceptualization, B.S. and S.K.S; Methodology, B.S. and S.K.S.; Software, A.K.G.; Validation, A.K.G. and C.P.; Formal Analysis, B.S., A.K.G. and S.K.S; Investigation, S.N.R; Resources, S.N.R and S.K.S; Data Curation, A.K.G. and S.S.M.; Writing – Original Draft Preparation, B.S. and A.K.G.; Writing – Review & Editing, B.S., A.K.G. and S.K.S; Visualization, A.K.G. and S.N.R.; Supervision, B.S. and S.K.S.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

Data are available on logical request.

Ethics approval

Ethical approval was obtained from the Institutional Animal Ethical Committee (IAEC) of IMS, Banaras Hindu University (Dean/2010-11/173 dated 23/07/2010).

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