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ASSESSMENT OF WOUND HEALING ACTIVITY OF A TOPICAL HERBAL GEL IN AN EXCISION MODEL OF ALBINO WISTAR RATS

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Abstract

There are several promising preclinical models, including mice, rats, rabbits, and pigs, that can be utilized to initiate acute or chronic wounds. These models can be induced using various distinct techniques, with excision being the most common. Once a suitable model has been determined fora study, researchers must select an appropriate and reproducible technique that enables the monitoring of wound improvement over time. This study analyzed the healing efficacy of ATC Topical herbal gel in Wistar rats using the excision wound model. The ATC Topical herbal gel was prepared at concentrations of 1%, 3%, and 9%, respectively, along with standard drugs (Povidone-Iodine Ointment USP 5% w/w-PI). The wound area contraction was measured on the 1st, 5th, 15th, and 21st days. The treated group exhibited significant changes in the improvement of wound healing and epithelialization, as indicated by biochemical biomarkers such as SOD (Superoxide dismutases), GSH (Reduced Glutathione), CAT (catalase), and MPO (myeloperoxidase). Furthermore, the treated group demonstrated a well-organized dermis devoid of inflammatory cells compared to the disease control group. Additionally, this experiment aimed to examine cytokine levels following wound healing.

Keywords: Topical Herbal Gel, Excision Model, Wound Contraction, Interleukin-1-beta, Transforming Growth Factor-beta1

Introduction

Medical plants are essential in the treatment of a number of illnesses and disorders, such as they are for the treatment of hypertension, diabetes, wounds, burns, and inflammatory diseases. [1,2]. The globe has started using plant extracts as an alternative for synthetic medications because to the increasing tolerance of chemical-based medicines and their multiple detrimental health consequences. [3].This extraordinarily potent herb is produced and harvested in India, after which it

is exported to all nations. [4]. Normal epidermis is damaged and loss its capacity to protect itself.[5] Poorer countries are more likely toget fatal infections from untreated incisions because they are more likely to do so.At the location of a lesion that has not healed, inflammatory mediators are continually generated, resulting in pain and edema.When we treat wounds with artificial pharmaceuticals, it's a time-consuming, costly process with many sideeffects. Medicinal plants are commonly employed in therapeutic settings as active medicaments for wound healing due to their low cost, availability, nontoxicity, ease of use, and patient participation.[7].

The main purpose of this study was to formulate and determine the wound healing potential of various herbal extracts gel, which contains three different medicinal plant extracts such as *Curcuma longa*., *Tridex procumbens*,. azadirachta indica. These herbal extracts containing gel is prepared using a biodegradable gelling agent i.e., Carbopol 934 and other biocompatible excipients. The wound healing potency of gel was pharmacologically evaluated by excision wound model in albino Wistar rat and determined the wound contraction rate at specified days of study.

Material and Methods

Animals

Experimental animals were used after the approval of experimental protocol (IAEC-KSOP-2022-23-14) from the Institutional Animal Ethics Committee (IAEC) of KIET School of Pharmacy, Ghaziabad (UP), India (Reg. No.: CPCSEA Reg. No.1099/PO/RE/S/07/CPCSEA). Thirty Wistar albino rats (180-200g) were used in the study. Animals were quarantined for 7 days and acclimatized five days. All animals were housed under standard room temperature and relative humidity (23 ± 2°C, 60 ± 5%) with a 12 h light and dark cycle throughout the whole study. For the whole study, the animals have been fed a normal diet and water with *ad libitum*. Animals were divided in five groups comprises of six animals per group.

Drugs and Chemicals

Analytical grade chemicals, drugs, and standard reagents such as Ketamine (Themis Medicare Limited, Uttarakhand, India) and Xylazine (Indian Immunological Limited, Siddipet, Telangana, India), Povidone Iodine 5% w/w USP (Win-Medicine Pvt. Ltd), Methylparaben, Carbapol 934, Glycerol, propyl paraben, Propylene Glycol and triethanolamine are purchased from Loba Lab chemicals from M/s Gupta agencies other lab grade chemicals were purchased from the local market. Hydroxyproline estimation kit was purchased from Quickzyme Biosciences, Netherland. Nitroblue Tetrazolium (NBT), Methionine, Riboflavin, 3,3',5,5'-Tetramethylbenzidine (TMB), Metaphosphoric acid, o-phthaldialdehyde and N-ethylmaleimide were purchased from Sigma India Private Limited.All the chemicals such as, Ambala Cantt, Haryana and *Azadirachtaindi ca, Tridexprocumbens*and *Curcuma longa*extract purchased from Herbo-Neutral, Greater Noida, Uttar Pradesh-201308.

Experimental Method for Excision Wound Model

Wound was created on all the animals according to the modified method (Morton and Malone, 1972)(7). On day first, the animals were anaesthetized (Ketamine, 87 mg/kg and Xylazine, 10 mg/kg intra-peritoneal injection) and inflicted with excision wound. The skin of the dorso-lateral flank area was shaved with an electrical clipper then cleaned with 70% alcohol. The skin from the predetermined shaved area was excised to its full thickness to obtain a wound area of about 300 mm^2, on the dorsal thoracic region 1.5 cm from the vertebral column on either side. Homeostasis phase was achieved by blotting the wound with a cotton swab soaked in normal saline. The grouping of the animals in the excision wound model was as follows: Group -1 Disease Control (DC), Group -2 Standard Group, Animals were treated with PI, Group -3, ATC-1% Gel, Group -4

ATC-3% Gel, Group -5 ATC-9% Gel. All the animals were treated daily twice application of vehicle or PI or ATC gelformulations at the calculated dose of 0.8 mg/mm^2 based on excised surface area. The wound contraction was calculated by the following formula.

$$\% \text{ wound contraction} = \left[\frac{\text{Healed area}^{\circ}}{\text{Total wound area}} \right] \times 100,$$

$$(^{\circ}\text{Healed area} = \text{original wound area} - \text{present wound area}).$$

Method of Obtaining topical Herbal Gel

Topical herbal gel prepared from the *Azadirachta indica*, *Curcuma longa* and *Tidex procumbens* extract prepared in 1%, 3%, 9% formulation of topical herbal gel. Distilled water was added to the Carbopol 934 and left overnight. To this mixture, triethanolamine was added vigorously. In water bath with a temperature not exceeding 50 °C, the *Azadirachtaindica*, *Curcuma longa* and *Tridexprocumbens* extracts in a concentration of 1%, 3%, and 9% were added to prepare three formulations, Gel-I (1% w/w), Gel-II (3%w/w) and Gel-III (9% w/w), respectively. Separately dissolved methyl and propylparaben in water were also added to this gel. Propylene glycol and glycerol were mixed in a separate beaker and added to this gel. The remaining quantity of purified water was added, and the pH was drop-wise adjusted with triethanolamine.

Biochemical Estimations

On day 21, Epithelized skin was exercised from the wounded area. Before proceed further the exercised skin tissue was washed in phosphate-buffered saline (pH 7.0) and centrifuged under cold conditions. The transparent supernatant was assayed using a spectrophotometer and the levels of antioxidant enzymes like SOD, GSH, CAT and MPO are determined(10–12). A piece of skin under the healing processed area was analyzed for hydroxyproline content, which is the basic constituent of collagen. Tissues were dried in a hot air oven at 60-70°C to constant weight and were hydrolyzed in 6 N HCl at 130°C for 4 hrs. in a sealed tube(13).

Estimation of cytokines by ELISA

ELISA is a lab technique used to measure cytokines in tissues or liquid from cell cultures to understand wound progression; it involves collecting tissue, breaking it down, and using the resulting liquid for the assay. Important cytokines in wound healing studies include TNF-α, IL-6, IL-1β, and TGF-β1, which help assess wound healing and identify treatment improvements (14–16).

Histopathological Study

The dissected wound samples were processed using a tissue processor and embedded in paraffin wax. Thin sections (5-7 µm) were cut with a microtome and floated in a tissue floatation bath. These sections were then placed on glass slides, treated to remove the wax, and stained with hematoxylin-eosin. Using a compound light microscope and Axio-vision software, the stained sections were examined and documented for scars, inflammation cells, epidermal hyperplasia, neovascularization, hyperpolarization, fibroblastic aggregation, and ulcer scores.(17,18).

Statistical Analysis

Statistical Analysis Results are expressed as mean±SEM. The differences between experimental groups were compared by one-way/ or two-way Analysis of Variance (ANOVA) (control vs. treatment) followed by Dunette's Multiple comparisons test for a single time point and Tukey's

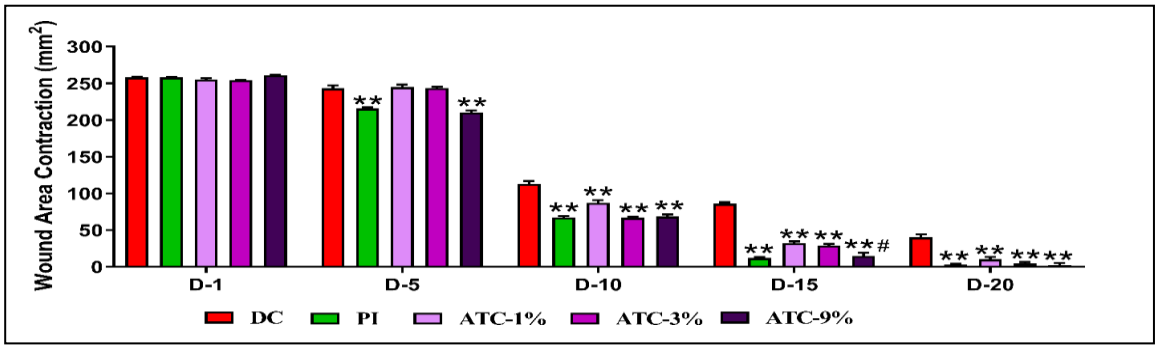
Multiple Comparisons Test for a multi-time point and were considered statistically significant when (*p< 0.05).

Results

Comparative studies have shown that ACT formulations (1%, 3%, and 9%) significantly reduce wound area contraction compared to standard medication, indicating their remarkable impact in promoting wound healing.

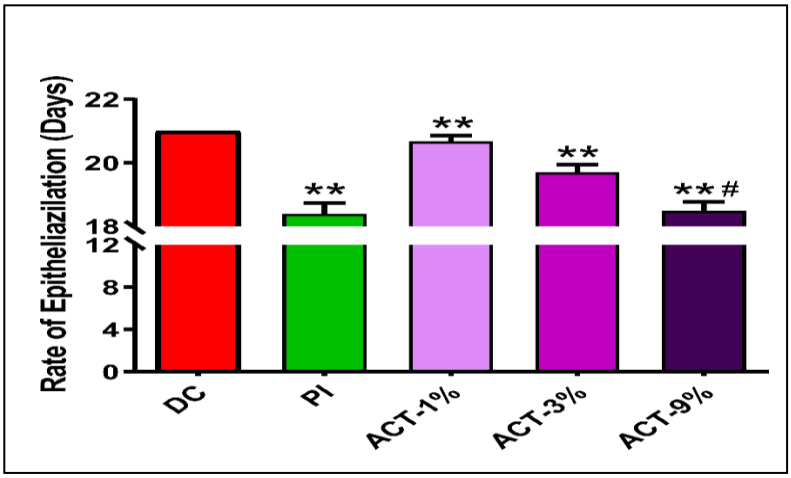
Wound Contraction

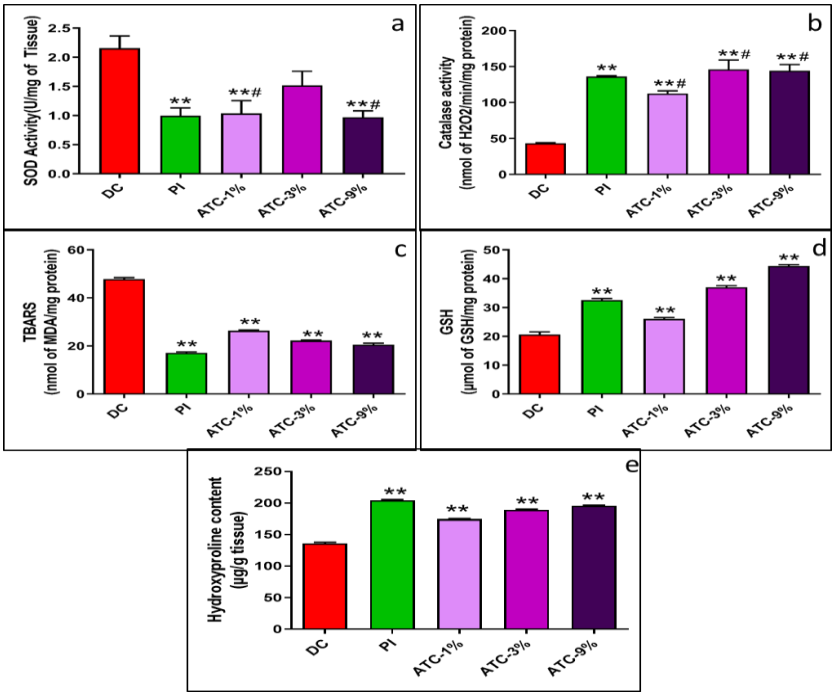
Wound area (measured in cm) was assessed on days 1, 5, 10, and 21. The initial 5 days exhibited an increase in wound size, followed by a gradual and progressive reduction. Notably, the wounds treated with ACT demonstrated accelerated healing. Comparative analysis revealed a significant enhancement in wound healing activity within the ACT-treated groups when compared to the disease control group (p <0.01), as illustrated in Figure 1.



Rate of Epithelization

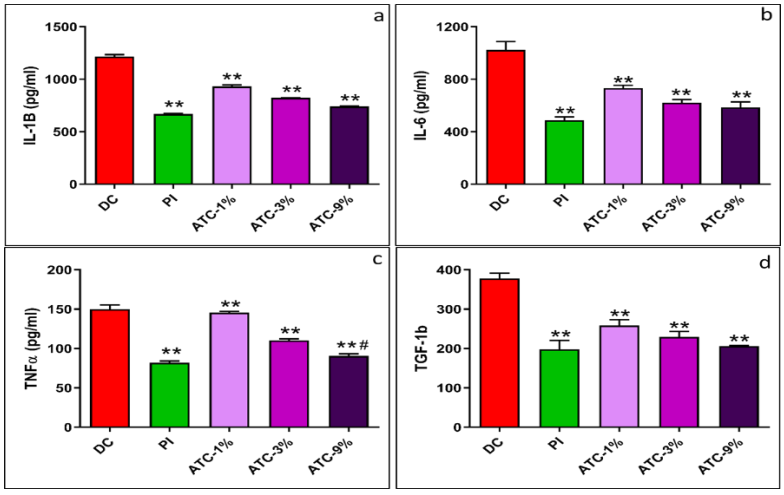
In Figure 2, a very high rate of closure of wound and epithelialization was observed post 18-19 days in treated groups. ACT Gel and Standard groups have shown significant wound healing activity (**p<0.01) and gradual closure of the wound by 19th day of post-surgery and by 21 days in control groups.





Cytokines Leve1

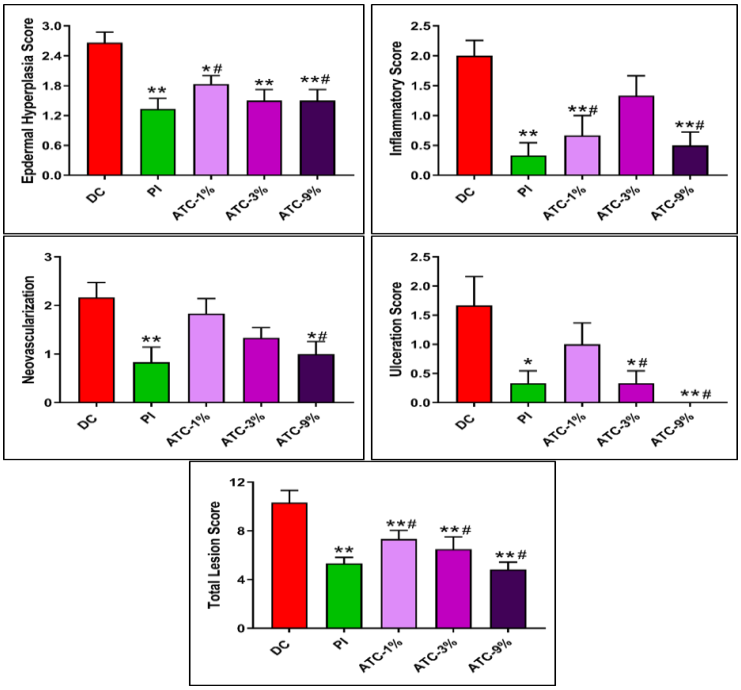
To understand the impact of ACT on cytokines, we assessed its effect on cytokine release. Neutrophils are attracted to wounds by cytokines, and they can also release cytokines that influence wound healing. ACT treatment significantly reduced cytokine levels compared to the control group, including IL-6, IL-1β, and TNF-α. Similarly, PI treatment significantly decreased the levels of these cytokines in 21-day skin tissue. ACT treatment also led to decreased production of TGF- β1 compared to the control group. These findings indicate that ACT has the ability to modulate cytokine release and potentially promote wound healing. See Figure 4(a-d) for graphical representation.



Histopathology and Microscopic Observation

Topical application of ATC leads to notable improvements in histopathological examination and microscopic analysis of wound healing. This includes enhanced wound contraction, increased collagen production, accelerated epithelization, and a well-structured dermis without inflammatory

cells, in contrast to the disease control group. Histological evaluation of rat wounds treated with ATC and the standard treatment demonstrates superior healing, depicted by reduced abrasion numbers, as shown in Figure-6 (A.(a-e), B. (a-f)).



Conclusion:-

Herbal extracts gel containing the extracts of *C. longa*, *T. procumbens*, and *azadirachta indica* wound healing in albino Wistar rats with a comparison of the synthetic formulation. The wound contraction rate was higher in the treatment of gel containing a higher concentration of extracts.

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Conflict Of Interest

The authors declare no conflict of interest.

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