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Influence Of Patola On Physiology Of Wound Healing In L929/ Mccoy Cell Line – An In Vitro Study

Kamath Nagaraj[1] , Nikhate Sunil[2]

[1]PhD Scholar, Sumandeep Ayurveda Medical College & Hospital, Sumandeep University (Deemed university) Vadodara Gujarat; Associate Professor, Department of Kriya Sharir, Sri Dharmasthala Manjunatheshwara College of Ayurveda & Hospital, Hassan.
[2]Professor & Head, Department of Kriya Sharir, Sumandeep Ayurveda Medical College & Hospital, Sumandeep University (Deemed university), Vadodara Gujarat
Corresponding Author email id: nagaraj.kamath1989@gmail.com

ABSTRACT

Background: Dosha, dhatu, and mala make up the foundation of the body. Under normal circumstances, each dosha has a unique site and will carry out its own tasks. Among the different processes occurring in the body, the physiology of wound healing is important. In everyday life, wounds remain a problem, with early and late complications presenting a frequent cause of morbidity and mortality. Vrana generally causes burning feeling that is similar to being burned by fire, bitten by ants, or being excised and incised by sharp objects. Tikta rasa is being helpful in treating loss of appetite, worms, thirst, toxicity, skin ailments, fainting, fever, excessive secretion of mucus and burning sensation. It pacifies kapha and pitta and causes Upashoshana of Kelda, Medas, Vasa, Majja. Laghu, Sheeta, Ruksha gunas promote vrana roopana. This study helps in determining the physiology of wound healing and its relation with tikta rasa, this study can serve as a platform to understand the relation between these two concepts.
Materials and Methods: The L929 / McCoy cell line will be procured from NCCS, Pune and sub cultured as per standard procedure. The 80-90 % confluent cell line will trypsinized and washed twice with Phosphate Buffered Saline (PBS) and cells are counted using a haemocytometer. Around 20,000 cells will be seeded to a 24-well plate supplemented with suitable medium with antibiotics and 10 % Fetal bovine serum and incubated for 24hrs at humidified CO2 incubator. After the incubation period old medium will be removed and washed twice with PBS. The experiment will be designed for Control, test group and positive control group. Using fine scalpel or needle uniform wound will be created on different groups on selected cell line. Different serially diluted test drug will be added to different groups and will be incubated for 48 to 72hrs to check the wound healing activity. After completion of incubation period medium will be carefully removed and washed twice with PBS and cells are fixed and stained using crystal /gentian violet stain. Plates are observed under microscope to check the activity of wound healing in different test groups.
Results: The results show Different concentrations of aqueous and hydro-alcoholic extracts of Patola has the effect on wound healing activity against L929/McCoy cell line.
Conclusion: The study shows the influence of Patola with the physiology of wound healing on the L929/McCoy cell line.

INTRODUCTION

Each person born on Earth undergoes pain starting from the moment the umbilical cord is severed. This is why Acharyas have emphasized the significance of wound healing [1]. The description of the management of wound is found in 'Vedas', which were written about 5000 years BC, exploration of wound healing documented in the Samhitas revealed significant insights [2]. Atreya Punarvasu, around 2000 BC, systematically expanded the understanding of Vrana (wounds) by detailing their causes, symptoms, and treatments. He was among the first physicians to catalogue a considerable array of remedies for wound healing. During the era of Acharya Sushruta, around 1000 BC, profound expertise in wound care was achieved. Sushruta, a skilled surgeon, comprehended the significance of wound care and extensively addressed numerous clinical aspects of wound healing. His contributions encompassed elucidation on the causes, classification, symptoms, prognosis, treatment, and complications of wounds. Vagbhata, an ancient physician from the 5th century AD, contributed to the understanding of wound healing by incorporating his own observations

into existing treatises, originally outlined by Charaka and Sushruta. They categorized wounds based on their stages and recommended various drug formulations and treatment methods.[3] The primary approach in treating severe types of wounds involve transforming contaminated wounds into clean ones. This comprises a range of methods, including regulating imbalanced dosha, addressing inflammation, performing surgical procedures, and managing factors that cause wounds. Vimlapana, Avasechana, Upanaha, Patanakriya, sodhana, Ropana and vaikritapaha are some traditional approaches used towards the management of Vrana. Ayurveda also recommends medications with properties such as Deepana-pachana, balancing dosha, krimighna, and vishaghna. It suggests that a combination of shodhana followed by ropana can effectively treat wounds. Drugs which possess Katu, Tikta, Madhura and Kashaya Rasa offer beneficial effects in Vrana.

The term 'Rasa' denotes taste, but it encompasses broader concepts such as Rasa Dhatu and Parada as well. Specifically, when perceived through the tongue, it refers to the primary or main taste, known as Pradhana Rasa. The action of taste occurs promptly upon exposure to the taste buds on the tongue. Taste serves as the distinctive sense that identifies the sweet, sour, bitter, salty, or umami qualities of a dissolved substance, facilitated by taste buds located on the tongue. These taste buds, the actual organs of taste, comprise numerous receptor cells, with approximately 10,000 taste buds present in humans. Taste buds, situated on the tongue and various regions within the mouth, possess taste receptor cells capable of discerning various tastes through the interaction of molecules with receptors on cell membranes. This perception of taste is affected by elements such as smell, texture, and temperature. Taste perception happens when chemical molecules from food reach the microvilli located at the apex of taste receptor cells (TRCs) within onion-shaped taste buds. [4]

Tikta, which translates to bitter, typically possesses antibacterial, anti-inflammatory, and antipyretic characteristics. It aids in treating anorexia by stimulating the production of digestive secretions and enzymes, alleviates symptoms like nausea, burning sensations, and various skin ailments, and facilitates wound healing. [5] Tikta Rasa alleviates Pitta dosha due to its sheeta and ruksha guna, which are contrary to the characteristics of Pitta dosha. It also increases Vata dosha because of its roksha and laghu guna. Tikta Rasa aids in digestion (Pachana), alleviates Kapha dosha and helps in Vranaghna, Puyaghna, Medohara respectively. [6] Patola, which has tikta rasa commonly recognized as the Sponge Gourd tree, holds a prominent place in Ayurveda for its extensive healing properties, particularly in wound care. The term 'Patola' signifies its usefulness and role in maintaining health. [7]

To investigate the therapeutic impact of medications on wound healing, the scratch assay is frequently utilized as a standard in vitro technique. It's utilized to examine the collective movement of cells within a two-dimensional environment. In this technique, a gap devoid of cells is generated within a continuous cell layer either through physical displacement or by eliminating cells from the designated area via mechanical, thermal, or chemical means. The presence of this cell-free region prompts neighbouring cells to migrate and fill the gap. As the drug Patola has tikta rasa which helps in the mitigation of pitta and kapha, this study can serve as a platform to understand the relation between tikta rasa and physiology of wound healing.

METHODOLOGY

The research methodology is essential because properly executed actions, aligned with the plan, are more likely to produce valuable results. Developing and adhering to a feasible approach technique are vital stages in this process. The methodology for this study has been divided into the following sections for clarity and comprehension.

1. Pharmaceutical study
2. Analytical study
3. Experimental study

1. PHARMACEUTICAL STUDY

A) Drug Preparation and Extraction

The pharmaceutical study commenced with the collection of raw Patola (Sponge Gourd) from the herbal garden of Sri Dharmasthala Manjunatheshwara (SDM) College of Ayurveda & Hospital, Hassan, Karnataka. The raw material was subsequently authenticated by the Department of Dravyaguna at the same institution. To prepare the Patola churna, the collected drug was stored in a shaded, dry area, weighed, and processed into a coarse powder using a disintegrator at the Department of Rasashastra and Bhaishajya Kalpana. The resulting powder was then forwarded to the SDM Centre for Research in Ayurveda and Allied Sciences, Udupi, for further extraction.

For the aqueous extract, 5g of accurately weighed Patola churna was added to 50ml of sterile distilled water in a conical flask. The flask was sealed with a cotton plug and parafilm, then agitated in a laboratory orbital shaker overnight. The following day, the mixture was filtered, and the filtrate was concentrated by evaporating the solvent using a water bath. The hydro-alcoholic extract was prepared using a similar cold maceration technique: 5g of the

churna was added to a solvent mixture consisting of 25ml sterile distilled water and 25ml methanol. After overnight agitation in an orbital shaker, the extract was filtered and subjected to evaporation via a water bath to obtain the final concentrated extract.

2.ANALYTICAL STUDY:

Assessing the quality of manufactured product is essential for any pharmaceutical preparation. The final product's quality is directly influenced by the characteristics of the raw materials. Therefore, consistent criteria are needed to verify the authenticity of the raw drugs and the quality of the finished product. To address this, analytical evaluation of key parameters is necessary. These evaluations help identify subtle variations that may occur during manufacturing, which can lead to significant differences between batches.

In this study, physicochemical tests, phytochemical analysis, and High-Performance Thin Layer Chromatography (HPTLC) were employed to assess the quality of Patola churna based on standard protocol mentioned in lab guide for ASU drugs, CCRAS. The analytical evaluation of Patola churna was conducted using standardized protocols to establish a baseline for quality, purity, and authenticity. The process began with an organoleptic study, where the sensory characteristics were documented: the powder appeared brownish-green with a texture ranging from smooth to slightly coarse. It emitted a characteristically bitter and pungent aroma, and the taste was intensely bitter, serving as a primary indicator of the presence of active limonoids and the overall potency of the raw drug.

Physicochemical analysis focused on quantifying the drug's physical properties to detect potential adulteration. The pH value was determined using a calibrated meter to assess chemical stability, while Loss on Drying at 105°C measured the moisture content to ensure it remained within limits that prevent microbial degradation. The extractive values (water and alcohol soluble) were calculated to determine the percentage of active constituents extractable by different solvents. Finally, total ash and acid-insoluble ash tests were performed using a muffle furnace at temperatures up to 600°C to measure inorganic residues and ensure the absence of earthy matter like silica. The preliminary phytochemical screening involved a series of qualitative chemical tests to identify the secondary metabolites present in the extracts. The presence of alkaloids was confirmed via Wagner's reagent (reddish-brown precipitate), and flavonoids were identified by a color-to-colorless reaction with sodium hydroxide and dilute acid. Tannins were detected using ferric chloride, while the Salkowski test confirmed the presence of terpenoids. Additional tests verified the presence of glycosides, saponins (via the foam test), and phytosterols, collectively establishing a comprehensive chemical profile for the Trichosanthes dioica Roxb sample.

To finalize the evaluation, High-Performance Thin Layer Chromatography (HPTLC) was employed to create a sophisticated chemical fingerprint. A methanolic extract of the churna was applied to pre-coated silica gel plates and developed using a mobile phase of Toluene: Ethyl acetate: Formic Acid (1:1:0.1). The plates were visualized under multiple UV wavelengths (365nm, 540nm, and 620nm) both before and after derivatization with vanillin-sulfuric acid reagent. This instrumental analysis allowed for the precise recording of Rf values and densitometric scans, ensuring batch-to-batch consistency and the identification of specific chemical markers within the manufactured product.

3. EXPERIMENTAL STUDY

The L929 / McCoy cell line will be procured from NCCS, Pune and sub-cultured as per standard procedure. The 80-90 % confluent cell line will be trypsinsed and washed twice with Phosphate Buffered Saline (PBS) and cells are counted using a haemocytometer. Around 20,000 cells will be seeded to 24 well plates supplemented with suitable medium with antibiotics and 10 % Fetal bovine serum and incubated for 24 hrs at a humidified CO2 incubator. After the incubation period the old medium will be removed and washed twice with PBS. The experiment will be designed for Control, test group and positive control group. Using fine scalpel or needle uniform wounds will be created on different groups on selected cell lines. Different serially diluted test drugs will be added to different groups and will be incubated for 48 to 72hrs to check the wound healing activity. After completion of the incubation period the medium will be carefully removed and washed twice with PBS and cells are fixed and stained using crystal /gentian violet stain. Plates are observed under microscope to check the activity of wound healing in different test groups.

In vitro wound healing assay to evaluate the migration of cells was carried out with few modifications (Cappiello F. et al., 2018). The Connective tissue (L929) cell line was procured from NCCS Pune and sub-cultured at SDM Research Centre, Kuthpady, as per standard procedure. Around 70-80 % confluent L929 cell line flask was selected and its old medium was removed and washed twice with phosphate buffered saline (PBS). The cells were treated with 100 µl of 0.25 % trypsin and incubated at 37°C incubator for about 3 to 5 minutes. After incubation time the excess trypsin was removed using pipette and the floating cells were transferred to a new fresh 15 ml centrifuge tube containing fresh medium (DMEM+FBS) and centrifuged for 5 minutes at 800 rpm. After centrifugation the old medium was removed and the cells were washed with PBS and centrifuged for 5 minutes at 800 rpm. After centrifugation the PBS was removed carefully and cell pellet is re-suspended in fresh DMEM medium with 10% fetal

bovine serum. The cells were counted using haemocytometer. Around 50,000 cells were transferred to a 60 mm petri dish and incubated for 24 hours supplemented with standard medium (DMEM+FBS). After incubation time, a wound was created on the cell line using a sterile needle and the old medium was removed and cells were washed with PBS. One set of cells were treated with routine normal (DMEM + FBS) medium which was considered as the control group. Second set of cells were treated with cisplatin to check its role in wound healing.
Different concentrations of aqueous and hydro-alcoholic extracts of Patola were prepared in serum free medium and added separately to different petri dishes respectively and incubated for 48 to 72hrs to check the healing activity. After incubation time the old medium was removed carefully and the cells were washed twice with phosphate buffered saline. The cells were fixed with one ml of 4% formaldehyde followed by stained with 0.05% of Crystal / gentian violet solution and incubated for 20 minutes at room temperature. After incubation time the excess stain is carefully removed and the petri dishes were carefully washed with PBS and dried at room temperature. The area of wound before and area of healing after treatment was measured under inverted microscope (Motic AE31) using Motic software.

OBSERVATIONS AND RESULTS

PHARMACEUTICAL STUDY

The colour of Patola changed from green to yellowish-brown. After drying, powdering of Patola was easy. The pharmaceutical processing of Patola (Trichosanthes dioica Roxb) resulted in significant physical transformations and weight changes. Initially, 4,500g of fresh Patola was collected, which underwent a color change from vibrant green to a yellowish-brown during the drying process. Once dried, the material weighed 557g, indicating a high moisture loss. The final yield of Patola churna after disintegration and powdering was 440g, demonstrating that the dried ones were easily processed into a fine medicinal powder.

ANALYTICAL STUDY

Evaluating the quality of manufactured medicines is crucial, especially given the variability of herbal ingredients influenced by multiple factors. Therefore, it is vital to establish consistent standards for verifying the authenticity of pharmaceutical raw materials and assessing the overall quality of the product.

Organoleptic characters

The quality of the manufactured churna was verified through sensory and chemical parameters. Organoleptically, the product was identified as a brownish-green powder with a pungent odor, a Tikta (bitter) taste, and a smooth texture upon touch. Physicochemical screening revealed a near-neutral pH of 6.8, while the loss on drying was 9.63%, suggesting appropriate moisture control for shelf stability. The total ash value was 9.66%, with a low acid-soluble ash of 1.42%, indicating minimal inorganic contamination. Extraction studies showed a higher yield in water, with a water-soluble extractive value of 11.05% compared to an alcohol-soluble extractive value of 7.55%.

Phytochemical screening

Comparative phytochemical analysis was performed on both aqueous and methanolic extracts to identify bioactive secondary metabolites. Both extracts tested positive for alkaloids, flavonoids, phenols, terpenoids, and phytosterols. However, solvent-specific differences were observed: tannins and saponins were exclusively present in the aqueous extract, whereas glycosides were only detected in the methanolic extract. This indicates that the choice of solvent significantly influences the profile of phytochemicals recovered from the Patola.

HPTLC of Methanol extract of Sponge Gourd

HPTLC analysis provided a detailed chemical fingerprint of the methanolic extract of Patola. Using a solvent system of Toluene: Ethyl acetate: Glacial acetic acid (5:5:1), the chromatography revealed distinct bands under short UV, long UV, and post-derivatization. A significant marker, Epicatechin, was identified at an Rf value of 0.19, showing prominent fluorescence at 365nm. Densitometric scans further quantified the chemical distribution across the plate at wavelengths of 540nm and 620nm, establishing a standardized profile for batch verification.

EXPERIMENTAL STUDY:

Observations on scratch assay of Aqueous extract: (Table no.1)

Sl.no	Drug/ Concentration	Wound margin	Cell migration	Cell density
1.	Control	Visible	Less	Low

2.	10 µg / mL	Visible	Less	Medium
3.	100 µg / mL	Visible	Average	Medium
4.	1000 µg / mL	Not visible	Good	High (Compact)
5.	5000 µg / mL	Visible	Good	High (Compact)
6.	10000 µg / mL	Visible	More	Very High
7.	Cisplatin mg/ml	Visible	-	-

Observations on scratch assay of hydro-alcoholic extract: (Table no.2)

Sl.no	Drug/ Concentration/HAE	Wound margin	Cell migration	Cell density
1.	Control	Visible	Less	Low
2.	10 µg / Ml	Visible	Less	Less
3.	100 µg / mL	Visible	Less	Less
4.	1000 µg / mL	Partially Visible	Good	Medium
5.	5000 µg / mL	Not visible	Good	High (Compact)
6.	10000 µg / mL	Not visible	More	Very High
7.	Cisplatin mg/ml	Visible	-	-

RESULTS:

The final results of the experimental study indicate that both the aqueous and hydro-alcoholic extracts of Patola possess significant wound-healing potential, as evidenced by the complete closure of the induced scratches in the L929 cell line. Across all tested concentrations—ranging from a minimal dose of 10 µg/mL to a maximum of 10,000 µg/mL—the initial wound sizes, which measured between 0.0001 mm and 0.0003 mm, were successfully reduced to 0 mm following the treatment period. This suggests that the bioactive constituents in Patola effectively stimulate the migration and proliferation of connective tissue cells, facilitating total gap closure regardless of the solvent used for extraction. In contrast, the Control group and the Cisplatin group displayed markedly different outcomes. While the untreated control cells eventually achieved closure through natural migratory processes, the cells treated with Cisplatin (mg/mL) showed a total arrest in healing activity. Specifically, the initial wound size of 0.0001 mm remained unchanged at 0.0001 mm after the addition of Cisplatin in both the aqueous and hydro-alcoholic experimental sets. This lack of movement highlights the inhibitory or cytotoxic nature of Cisplatin on cell migration, further emphasizing the comparative efficacy of the Patola extracts in promoting tissue repair.

Table 3: Wound healing activity of aqueous and hydro-alcoholic extracts of Patola against L929 cell line.

Concentration of drug	Wound size before treatment (AQ group)	Wound size after addition of aqueous extract of Patola (AQ group)	Wound size before treatment (HY-AL group)	Wound size after addition of hydro-alcoholic extracts of Patola (HY-AL group)
Control	0.0002 mm	0 mm	0.0002 mm	0 mm
10 µg / mL	0.0002 mm	0 mm	0.0002 mm	0 mm
100 µg / mL	0.0003 mm	0 mm	0.0001 mm	0 mm
1,000 µg / mL	0.0001 mm	0 mm	0.0002 mm	0 mm
5,000 µg / mL	0.0001 mm	0 mm	0.0001 mm	0 mm
10,000 µg / mL	0.0002 mm	0 mm	0.0002 mm	0 mm
cisplatin (mg/ml)	0.0001 mm	0.0001 mm	0.0001 mm	0.0001 mm

DISCUSSION

Patola contains bioactive chemical compounds like vitamins A and C**,** saponins**,** tannins**,** trichosanthin, and essential fatty acids including linoleic and oleic acids, Vitamin A, Vitamin C, Nicotinic acid, Riboflavin, Thiamine, 5-hydroxytryptamine, and free carbohydrates. Patola contains triterpenoids, including bitter cucurbitacin glycosides, tetracyclic and pentacyclic triterpenes (such as lupeol, betulin, and taraxerol), and cucurbita-5,24-dienol.[8]. These triterpenoids belong to a broad category of terpenoids characterized by a 30-carbon chain structure, formed from six linked isoprene units arranged either in a head-to-tail or non-head-to-tail sequence. [9] Bioactive compound limonoid, which is a key active ingredient is a type of Furano lactone. Limonoid is particularly noted for its anti-inflammatory properties and role in activating the body's own opioid pathways. In experiments with damaged rat paws, Soares, it is observed that Sponge Gourd-derived limonoid effectively reduced oedema and moderates the formation of fibrovascular tissue. This effect was attributed to its specific inhibitory action on critical inflammatory molecules such as tumour necrosis factor-alpha (TNF-α) and various interleukins (ILs). Also, another noteworthy compound epoxy-azadiradione exhibits anti-inflammatory effects. This compound shows cytotoxic potential in various diseases by modulating the macrophage migration inhibitory factor (MIF). It inhibits MIF's tautomeric activity and blocks the translocation of nuclear factor-kappa B (NF-kB), thereby preventing the release of cytokines like IL-1α, IL-1β, IL-6, and TNF-α from proinflammatory macrophages.[10]

The pH of Patola churna was 6.8. The loss on drying of Patola churna is 9.63% this indicates total moisture content present in the sample. The total ash present in Patola churna was 9.66% indicates the presence of inorganic matter in the sample. Acid-insoluble ash was 1.42 %. Total amount of water-soluble extractive was about 21.05%. The Total amount of alcohol-soluble extractive was about 14.55%. The amount water soluble extractive is higher when compared to alcohol soluble extractive. The percentage of foreign matter is zero as the drug was collected fresh from herbal garden.

Except for the Acid-insoluble ash all other parameters are under standard limit mentioned in The Ayurvedic Pharmacopoeia of India. Acid insoluble ash is bit higher than standard limit may be due to the presence of silica and earthy materials in drug. Preliminary phytochemical analysis done for aqueous extract of Patola churna showed presence of Alkaloids, Flavonoids, Phenols, Tannins, Terpenoids, Saponins, Phytosterols whereas that of alcoholic extract shows Alkaloids, Flavonoids, Phenols, Terpenoids, Glycosides, Phytosterols. Alkaloids can modulate inflammation by enhancing chemotaxis of macrophages and neutrophils to the wound site, which is crucial for the inflammatory phase of healing. It also exhibits antimicrobial properties, which help prevent infection in wounds—an essential factor for successful healing. By reducing oxidative stress at the wound site, alkaloids help protect cells from damage during the healing process. This antioxidant activity is vital for maintaining cellular integrity and facilitating repair mechanisms [11]

Flavonoids by modulating the expression of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α. helps in controlling the inflammatory response during the healing process. These compounds scavenge reactive oxygen species (ROS), mitigating oxidative stress that can hinder wound healing. By enhancing the body's antioxidant defences, flavonoids protect cells from damage and promote a conducive environment for healing. It also promotes the proliferation and migration of key cells involved in wound healing, such as fibroblasts and keratinocytes. This activity is essential for re-epithelialization and collagen synthesis, which are critical for restoring the integrity of the skin.[12]

Phenols can enhance collagen synthesis, which is essential for restoring the integrity of the skin. Increased collagen deposition provides structural support and facilitates the wound healing process. These compounds have demonstrated efficacy in promoting wound closure, reducing scar formation, and accelerating the healing process in various in vitro and in vivo studies. It also stimulates the proliferation and migration of cells involved in wound healing, such as fibroblasts and keratinocytes. They also promote angiogenesis, which is critical for supplying nutrients and oxygen to the healing tissue. Phenols also have anti-inflammatory and anti-oxidant properties which may provide favourable environment for healing.[13]

Tannins enhance the proliferation of epithelial cells, facilitating faster re-epithelialization of wounds. This is particularly important for restoring the integrity of the skin barrier. Studies have shown that tannin treatments result in better quality eschar and reduced scar tissue formation compared to conventional treatments. Tannins have been incorporated into various topical formulations, including ointments and dressings. Their ability to form complexes with proteins aids in creating protective barriers over wounds while delivering therapeutic effects.[14] Terpenoids possess antimicrobial properties that can prevent infections in wounds, a significant factor that impedes healing. In vivo studies have demonstrated that terpenoids can accelerate wound contraction, an important indicator of epithelialization and the start of the healing process. Wounds treated with terpenoids showed a significantly higher contraction rate compared to untreated controls.[15] Sponge Gourd contain flavonoid glycosides such as Apigenin-7-O-glucoside and Quercetin glycosides etc. Glycosides enhance the proliferation and migration of key cells involved in wound healing, such as fibroblasts and keratinocytes. This is vital for re-epithelialization and tissue remodelling. Flavonoid glycosides like apigenin-7-O-glucoside and quercetin glycosides contribute to the antioxidant and free

radical scavenging properties of Sponge Gourd. Many glycosides exert anti-inflammatory actions that help mitigate the inflammatory phase of wound healing, allowing for a smoother transition to the proliferative phase.[16]
Saponins have surfactant properties, allowing them to form stable foams in aqueous solutions. Saponins exhibit antibacterial and antifungal activities, making them effective against various pathogens. This property may help treating infections. It also modulates inflammatory responses, which is crucial in the treatment of inflammatory diseases and conditions. The presence of saponins in Sponge Gourd may contribute to wound healing processes by promoting tissue regeneration and reducing inflammation at the injury site. The antioxidant capacity of saponins may aid in the protection against oxidative stress and cellular damage.[17] Phytosterols by inhibiting inflammatory pathways reduces the production of inflammatory mediators. Sponge Gourd phytosterols scavenge free radicals and protect cells from oxidative damage.[18]
The prepared churna (Methanolic extract) was subjected to HPTLC fingerprinting at different UV region wavelengths (365nm, 540nm and 620nm). HPTLC and densitometric scan revealed number of peaks. The color spots observed indicates the presence of different components in the sample. It acts as fingerprint of the used sample, which can be taken as reference for the preparation of churna. At 365nm, 540nm and 620nm, 11, 6 and 6 peaks were found with different retention factor starting from 0.01 to 0.94, 0.00 to 0.95 and 0.00 to 0.95 respectively. The hands at different Rf value indicate the presence of particular active compounds in Patola churna.
Vrana can be correlated to wound on the basis of literary review, in which the causative factors, occurrence, symptoms are similar in both the conditions. In the present study, wound healing activity of aqueous (AQE) and hydroalcoholic (HAE) extract of Patola churna was assayed on L929 cell line by causing uniform wound with the help of needle. The concentration of test samples used are 10 µg/mL, 100 µg/mL, 1000 µg/mL, 5000 µg/mL and 10000 µg/ml which were introduced or added into petri dishes containing cell line and allowed to act for 72hrs. The control group indicates a baseline with limited cell migration and low density, reflecting the natural healing process without any treatment.
At 10 µg/ml of AQE, there's a slight increase in cell density, but migration remains limited, suggesting that this concentration may not be sufficient to enhance healing. 100 µg/ml concentration of AQE improves cell migration to an average level which may indicate a potential positive effect on healing compared to lower dose, but visible wound margin may indicate less cell proliferation.
1000 µg/ml concentration of AQE shows wound healing, with a compact cell density and good migration. The fact that the wound margin is not visible may suggest a strong effect on cell proliferation and migration. Similar to the 1000 µg/ml concentration, 5000 µg/ml of AQE dose promotes good migration and high density, but the visible wound margin may indicate some limits in complete wound closure compared to the 1000 µg/ml. At high concentration of 10000 µg/ml of AQE, there's an increase in migration and density, suggesting a strong stimulatory effect but the wound margin is visible. This could indicate that, despite some positive effects, the concentration might be too high for optimal cell behavior, possibly leading to issue like overpopulation of cells that could hamper complete closure of the wound.
At 10 µg/ml and 100 µg/ml of HAE, there is minimal migration, reduced cell density and visible wound margin which may suggests that the drug does not significantly promote healing.1000 µg/ml of HAE shows a marked improvement, with good migration and medium density. The partially visible wound margin may suggest that the cells are starting to close the wound effectively. At 5000 µg/ml of HAE, the wound is completely closed, and there is high cell density with good migration. This indicates an optimal effect on cell proliferation and migration, which may facilitate effective healing. 10000 µg/ml concentration of HAE also results in a closed wound but more migration and very high cell density which may give raise to overpopulation of cells. As Cisplatin is an antiproliferative drug it shows adverse effects on L929 cell behavior, with visible wounds and no migration or density.

CONCLUSION

Tikta rasa has relation with physiology of wound healing on L929/McCoy cell line in terms of increase in cell migration and cell density, reflects the reduction in wound margin.

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