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EFFECT OF POLYHERBAL *CONTAINING CENTELLA ASIATICA* AND *ELETTARIA CARDAMOM* ON WOUND HEALING IN RATS

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ABSTRACT

In this study, we formulated and evaluated the pharmacological effect of methanolic extract of Centella Asiatica and Elettaria Cardamom on wound healing in rats. The present study focuses on the study of the wound healing activity of the combined poly-herbal formulation of the *Centella Asiatica* and *Elletaria cardamom* in the following study animals were divided into three group in which group 1 was control which was treated with normal cream base, second group was test group which was treated with combined poly herbal formulation of *Centella Asiatica* and *Elettaria cardamom* extract and another group was standard group which was treated with synthetic market ointment. The treated, standard groups are compared with control group and values are represented as the mean± SEM; n=3; P<0.05;P<0.01 Vs control. The following study was found that there is a significant decline in affected wound area. On the other side significant improved texture of animal's hair found after wound healing.

INTRODUCTION

Wound is a break in the epithelial integrity of the skin and may be accompanied by disruption of the structure and function of underlying normal tissue and may also result from a contusion, hematoma, laceration or an abrasion. Wound healing starts from the process of injury and can persist for varying periods of time depending on the level of wounding and the process can be broadly categorized into three stages; inflammatory phase, proliferate phase, and finally the remodeling phase which ultimately determines the strength and appearance of the healed tissue. Wound care and maintenance involves a number of measures including dressing and administration of painkillers, use of anti-inflammatory agents, topical systemic antimicrobial agents and healing promoting drugs. **Wound** is defined as disturbance of cellular, anatomical, and functional continuity of a living tissue. It can be produced by physical, chemical, thermal, microbial, or immunological insult to the tissue. When skin is torn, cut, or punctured it is termed as an open wound and when blunt force trauma causes a contusion, it is called closed wound, whereas the burn wounds are caused by fire, heat, radiation, chemicals, electricity, or sunlight[1].

The main purpose of medical care for wound is to preserve functions. The main objective of this study is to formulate the herbal medication which is containing elettaria cardamom and centella asiatica, both these herbal drugs are compatible with each other and contain good wound healing effects. The formulation of this blend is a noval idea because on the basis of previous study cardamom exhibits anti inflammatory activity and centella asiatica posses wound healing activity so the blend of both the drug accelerates wound healing along with the diminishing of inflammatory reactions.

TYPE OF BURNS

Thermal Burns: Thermal burns occur when you come in contact with something hot. Typically, you will suffer a thermal burn when you touch:

- Flames or fire
- Hot, molten liquid or steam (referred to as a scald)
- Hot objects, such as cooking pans, irons, or heated appliances.

Chemical Burns: You may receive a chemical burn if your skin and/or eyes come in contact with a harsh irritant, such as acid. Substances that cause chemical burns include:

- Chlorine
- Ammonia
- Bleach
- Battery acid
- Strong or harsh cleaners

Electrical Burns: Electrical burns arise when the body comes in contact with an electric current. Our internal systems are not resistant to electricity, so you may be injured if a strong jolt enters your body.

Friction Burns: A friction burn can occur when skin repeatedly rubs against another surface or is scraped against a hard surface. Like other burns, friction burns are categorized into degrees**.**

Radiation Burns: Cancer patients undergoing radiation therapy may suffer from an injury known as radiation burn. High-energy radiation is used to shrink or kill cancerous cells, and when it passes through the body, skin cells may be damaged. If you're frequently receiving radiation treatments, your skin cells may not have enough time to regenerate, and sores or ulcers may develop.

BURN WOUND

A burn is type of injury that can be caused by heat, cold, electricity, chemical, light Radiation or friction. Burn can be highly variable in terms of tissue affected, the severity and resultant complication. Muscle, bone, blood vessel and epidermal tissue may all be damaged with succeeding pain due to deep injury to nerves. Burn injury produces profound systemic changes such as oligemic shock, anemia, renal failure and metabolic disturbance. It causes direct tissue damage as well as inflammatory reaction. Infection is another major complication of thermal injury. It also leads to increased oxidant stress in the cells as seen by decreased endogenous non enzymatic and enzymatic antioxidant activity. The mediators of burn shock include histamine, serotonin, kinins, oxygen free radical, prostaglandins, thromboxane and interleukins. After a severe burn, the injured surface becomes vulnerable to the bacteria, due to loss of protective skin barrier. High levels of bacteria in the burn wound can decrease the availability of growth factor, which can retard the healing process. Slow healing and non healing wounds, such as ulcers, as well as wounds caused by major or minor injuries, surgery, or burns, represents the most widespread treatable condition encountered by humans and animals. Wound repair is a well highly coordinate process that involves a series of overlapping phases: inflammation, cell proliferation, matrix deposition and tissue remodeling. Underlying repair is a complex dynamic series of events including clotting, inflammation, cell proliferation, matrix deposition and tissue remodeling. Underlying repair is a complex dynamic series of events

including clotting, inflammation, granulation tissue formation, epithelization, revascularization, collagen synthesis and wound contraction. One of the plants used in dermatology is Centella asiatica (L.) Urban, synonym Hydrocotyle asiatica L. is from the family Apiaceae, also known by the common name Gotu kola or Indian pennywort. It is a topical creeping plant and embrace a variety of chemical constituents namely –asiatica acid, madecassic acid, asiaticoside, madecassoside etc. It grows in the tropical regions of Asia, Oceania, Africa and America. C. asiatica herb is recommended in the treatment of dermatoses and skin lesions such as excoriations, burns, hypertrophic scars or eczema as well as in non-dermatological diseases like gastric ulcers, gastric mucosal lesions[2] anxiety[3] and for improving cognition in neurodegenerative disorders[4]. C. asiatica has also been found beneficial in chronic venous insufficiency, mainly by improvement of microcirculation[5] C. asiatica extract (International Nomenclature of Cosmetic Ingredients, INCI) is used also as an ingredient of cosmetics[6]. The present study focuses on the study fn the wound healing activity of the poly-herbal formulation of the centella asiatica and elletaria cardamom.

BURN CLASSIFICATION

Now that you are able to identify the different types of burns, you should know how burns vary. Burns are classified by degree: first, second, and third.

First-Degree Burns**:** First-degree burns damage the outer layer of your skin, better known as the epidermis. Typically, these burns heal themselves within a week. A good example of a first-degree burn would be sunburn. So, don't forget you're tanning lotion.

Second-Degree Burns**:** Second-degree burns damage both the outer layer and the layer beneath it, which is called the dermis. With these burns, a skin graft is sometimes needed. A skin graft is essentially a procedure in which healthy skin is transplanted to cover the burn, allowing it to heal.

Third-Degree Burns

Third-degree burns are more severe, as they destroy both layers of your skin. Hair follicles, sweat glands, and other tissues tend to result in damage as well. Remember those skin-grafts we talked about? They are always required with third-degree burns.

STAGES OF WOUND HEALING

The last few years have seen a renewed focus on the use of animal models to investigate the mechanisms of wound healing. Wound healing is a very complex and intricate process. This review is concerned with the repair of wounds in skin; we will not attempt to deal with the molecular factors involved in the healing process. In most species, the normal response to trauma occurs in three overlapping but distinct stages: inflammation, proliferation, and re-epithelialization/ re-modeling [7-9]. The immediate response to injury is mediated by damaged cells along the wound site. These cells transmit "stress" signals immediately to activate the inflammatory response. The priority of the inflammatory responses is to counteract microbial wound infections and this takes precedence over wound closure. During this phase, pro-inflammatory factors like serotonin, bradykinin, prostaglandins, prostacyclins, thromboxane, and histamine are released into the local wound site. The goal of this initial phase is to re-establish tissue integrity and homeostasis. Once the necessary framework has been accomplished in the inflammatory phase, the subsequent production of a new functional barrier is initiated in the proliferation phase [9]. The infiltration of the wound site by fibroblasts and other cell types initiates the proliferative phase. The function of fibroblasts is primarily collagen deposition in the dermal wound area [7-9]. Increased production of Type III collagen and fibronectin occurs within the first 3 days after tissue injury. This

activates several signaling pathways that modulate healing. Fibroblasts also secrete cytokines that attract keratinocyte cells to the injury site. The keratinocytes function in re-epithelializing the wound site, ultimately restoring the barrier function of the epithelium [8-9]. Concurrently with fibroblast and keratinocyte migration, angiogenesis also occurs. Angiogenesis, the formation of new blood vessels, is critical for wound healing since fibroblasts and epithelial cells require a continuous supply of oxygen and nutrients to function optimally (9). The proliferative stage terminates with the breakdown of provisional extracellular matrix leading to a decline in hyaluronic acid and an increase in chondroitin sulfate, which gradually triggers the fibroblasts to stop migrating and proliferating [7]. In the final stage of wound repair, the remodeling stage, collagen undergoes cross-linking to improve its strength and stability. However, as remodeling progresses, collagen synthesis and collagen catabolism begin to take effect [9]. Imbalance in either excessive matrix synthesis or decreased matrix catabolism can lead to keloid and hypertrophic scar formation. As the extracellular matrix is reorganized and remodeled, newly formed blood vessels continue to mature and form functional vascular networks. Depending on the wound size, the remodeling phase can last anywhere from weeks to years [9].

FACTORS INFLUENCING HEALING

Two types of factor influencing the wound healing

1. Local factors: includes

- Infection by tissue organization which delay healing
- Poor blood supply which shows healing
- Movement of affected part of delay healing
- Exposure to ionizing radiation delay granulation
- Exposure to ultraviolet light facilities healing
- Foreign bodies including sutures interferes in healing

2. Systematic factors: includes

- Wound healing is rapid in young and slow in aged people
- Nutritional deficiency of vitamin C and zinc delay healing
- Haematological abnormalities also affects healing
- Diabetics are more prone to infection and hence delay healing
- Administration of glucocorticoids (anti-inflammatory) delay healing. (11).

MATERIAL AND METHODS

PREPARATION OF EXTRACT *(Centella Asiatica:* Whole plants of *Centella asiatica* were collected during month of September-October from the campus of the M.J.P. Rohilkhand University. Uttar pradesh and were identified by the Department of Plant science. After collection the plants were washed with distilled water and dried in the ventilated shed area in the lab. Air dried whole plants were crushed into 40 mess size for the extraction. The crushed plants were soaked in methanol for 48 hours, decanted, filtered through muslin cloth and Whatman's filter paper No. 1. The filtrate was concentrated by evaporating methanol by distillation process at 50-600C. The residue obtained after removing the solvent was transferred to a petri dish and kept over water bath at 40-500 C till the solvent was completely evaporated. After complete removal of solvent it was stored at 40C for future use **(17)**.

PREPARATION OF EXTRACT *(Elletaria Cardamom)*: Dry *Elettaria cardamomum* seed will be purchased from the market. The plant seed will be identified by botanist at M.J.P. Rohilkhand University. A voucher specimen of the plant material will be deposited at the herbarium of botany department. The dried seed of plant (100g) will be grinded into fine powder using an electric grinder and extracted by percolation method and

through using methanol (80%) for 72 h at room temperature. The solvent will be removed in a rotary evaporator, and after filtering, the extracts were concentrated to dryness (18).

DEVELOPMENT OF POLYHERBAL FORMULATION: Ingredient of oil phase (A) was melted in a beaker by using water bath on constant stirring. Components of aqueous phase (B) were mixed together and warmed to about same temperature of oil phase (up to 700℃). The preservative methyl paraben and concentrated aqueous extract of the plants were added into aqueous phase and heated. Then oil phase was added to water phase little by little on constant stirring and perfume was added to it when the temperature was 350℃ - 400℃.

Two different formulations were prepared by using varying concentration of aqueous extract, stearic acid and liquid paraffin.8,21,22

BURN WOUND MODEL (HOT WAX MODEL): Healthy adult (Sprague Dawley) female rats are selected (150-200) were divided into 3 groups, each group consisting of 3 rats and each animals kept separately under laboratory condition. They had free access to commercial pallet diet and ad libitium. Each rat will be anesthetized with ketamine Hcl inj. (50 mg/kg) and the hair on the back will be clip with electric clippers. Burn wounds will be created by pouring hot molten wax at80C into a metal cylinder placed on the back of the rat. The metal cylinder has 300mm areas of circular opening and the capacity of to hold 4.0g of the wax. On solidification of the wax (8 min) the metal cylinder with wax adhered to the skin will be remove, which left distinctly demarked circular wounds of 300mm. After this each animal will be placed in a separate cage for full recovery from anesthesia before being returned to holding rooms (19).

Drug Treatment and Group Division

Group 1: Control (untreated) – base cream

Group 2: treated- pollyherbal firmulation

Group 3: standard- market ointment

Different Parameter Measurement Procedure

- **Wound Contraction:** The size of the wound will be measured by taking daily photo used digital camera transparent scale. by taking the initial size of the wound contraction using following equation =
- Initial wound size - specific day wound size x 100

Initial wound size

Wound contraction, which contributes to wound closure and restoration of the functional barrier. Contractions, which contribute to wound closure, were studied on alternate days from Day 1 to Day 9, i.e. starting from the day of operation till the day of complete epithelization by tracing the raw wound on a transparent sheet. Wound contraction was calculated as percentage of the reduction in original wound area size (20).

- **Determination of Period of epithelization:** Falling of scab leaving no raw wound behind was taken as end point of complete epithelization and the days required for this was taken as period of epithelization (21).
- **Measurement of wound area:** The progressive changes in wound area were monitored by a camera on predetermined days i.e., 2, 4, 8, 12, 16 and 20. Later on, wound area was measured by tracing the wound on a millimeter scale graph paper (22).

EVALUATION OF CREAM

1. **Determination of clarity and colour:** It was done with naked eyes against white background.
2. **Determination of odour:** It was done by mixing gel in water and taking the smell. ***Homogeneity*****:** Homogeneity of the creams was tested by visual observation and was ranked as follows:

+++ = Excellent, ++ = Very Good, + = Good, and - = Poor.

3. **Spreadability:** Spreadability of the creams was performed by applying the

cream on the skin and noticing whether spreading was good or not and was ranked as follows:

+++ = Excellent, ++ = Very Good, + = Good and - = Poor.

Homogeneity: The formulation was tested for homogeneity by visual appearance and touch

4. **Appearance:** The appearance of the cream was judged by its color, pearlescence and roughness and graded.
5. **After feel:** Emolliency, slipperiness and amount of residue left after the application of fixed amount of cream were checked.
6. **Type of smear:** After application of cream, the type of film or smear formed on skin were checked.
7. **Removal:** The ease of removal of the cream applied was examined by washing the applied part with tap water.

RESULT: The formulated wound healing cream was evaluated for several physicochemical tests and the results were shown in Table 3. The type of smear formed on the skin was not greasy after the application of both creams. The creams were easy to remove after application by washing with water. The formulations were able to produce uniform distribution of extracts in the cream. This was confirmed by visual examination and by touch. There were no changes in term of colour of the cream even it was kept for a long period of time. After feel test showed that the creams were emollient and slipperiness.

Effect of Centella asiatica and Elettaria cardamom extracts on healing of burn wounds

Burn wound lesions: On Day 6, the wound in the control (untreated) groups became swollen and bruised. In contrast, the wounds in all extract-treated groups showed a mild degree of swelling and the wound surface was rather dry. Most wounds in the extract-treated group had begun to contract from the wound edge. On Day 12, all wounds in the untreated groups were dark red, showed thickening of the skin at the wound site, and remained unchanged in size from the first day. Most of the wounds treated with the extracts showed wound contraction compared with the control group. The wounds in the extract treated group showed marked hair growth. Some wounds in the extracts treated groups had scabs covering the wound surface. On Day 24, all wounds in the untreated groups showed moderate exudation and no hair growth, with scabs covering the wound surface. All wounds in the extract-treated groups had a dry surface, progressive wound contraction, and increased hair growth. On Day 30, the last day of the experiment, all wounds in the untreated groups showed moderate exudation and scabs starting to separate from the wound surface. The wounds in the extracts-treated groups showed a marked reduction in size and continuous growth of hair at the wound site. The wounds in the extracts-treated groups showed the most marked reduction in wound size.

Degree of healing of burn wounds: The effects of Centella asiatica and Elettaria cardamom extracts on the degree of healing of burn wounds are shown in Table 4. The degree of wound healing did not differ significantly among the untreated (control) groups at every time point. On Day 6 after burn injury, only animals from the extracts-treated group had a higher degree of wound healing compared to the vehicle control group. Similar results were observed on Day 7. On Day 18 after burn injury, the degree of wound healing in animals treated with Centella asiatica extract and elletaria cardamom extract was significantly higher than that in the vehicle control group.

Table no: 1: Skin Histology of Rat[25]

Trait	Rat
Hair coat	Dence
Epidermis	skinny
Dermis	skinny
Panniculus carnosus	Present
Skin architecture	loose
Wound-healing mechanism	Contraction

Figure 1: Wound Healing Process

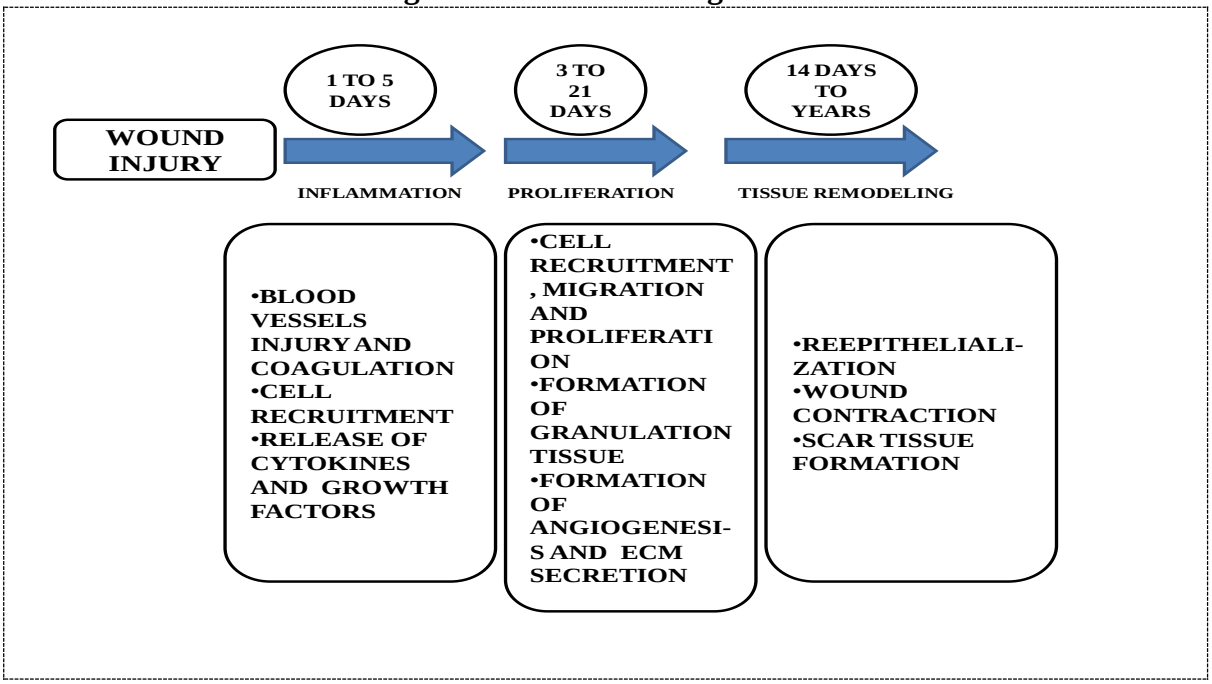

Table- No 2: Final Composition of Herbal Cream

S. no	Ingredients	Formulations in % w/w
1.	Aq. Extract of Centella Asiatica	3.0
2.	Aq. Extract of Elletaria Cardamom	2.0
3.	Aloevera	3.0
4.	White Petroleum	0.8
5.	Liquid Parafin	8.3
6.	Lanolin	0.8
7.	Stearic Acid	16.7
8.	Propylene Glycol	3.5
9.	Triethanolamine	1.0
10.	Tween 60	5.0
11.	Methyl Paraben	0.1
12.	Water	q.s

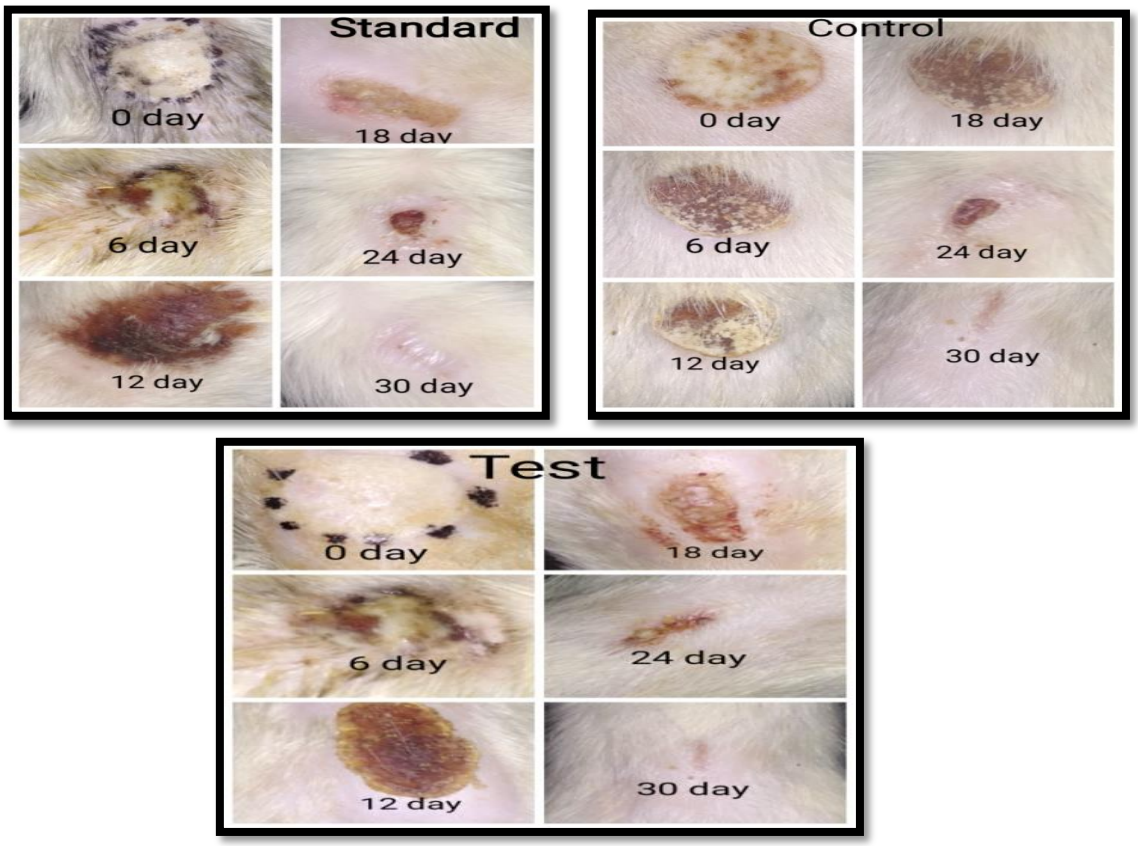

Table no: 3. Physicochemical Evaluation of the Formulated Wound Healing Cream

Parameters	Results
Clarity and transparency	Good
Odour	Good
Spreadbility	Good
Homogeneity	Good
Appearance	Brown Color
After Feel	Soft And Slippery
Type Of Smear	Mild Greasy
Removal	Easy

Table no: 4. Observation table of wood healing effects

Days/treatmens	0^{th} day	6^{th} day	12^{th} day	18^{th} day	24^{th} day	30^{th} day
(Batch-1)						
control	182.30±0.97	171.58±0.84	121.02±0.86	68.92±0.69	43.53±0.98	10.39±0.37
Test	181.35±0.91	142.68±1.37	78.56±0.85	32.17±0.67	17.43±1.02	4.24±0.40
standard	178.21±0.91	142.68±0.86	62.47±0.89	32.17±0.67	14.14±0.93	2.21±0.38
(Batch-2)						
control	184.25±0.95	170.28±0.86	120.89±0.81	65.99±0.65	41.86±0.89	11.02±0.33
Test	178.89±0.87	143.56±1.22	76.87±0.88	34.19±0.66	15.82±1.24	5.01±0.38
standard	172.36±0.82	140.25±1.05	65.16±0.78	38.18±0.78	13.69±0.87	2.56±0.40
(Batch-3)						
control	182.98±0.92	169.58±0.95	119.45±0.84	69.85±0.68	40.85±0.84	10.89±0.51
Test	180.41±0.92	141.66±1.32	71.87±0.52	28.98±0.61	15.23±1.24	4.89±0.54
standard	170.88±0.87	140.69±1.01	61.54±0.62	23.56±0.68	14.85±0.89	2.11±0.31

The treated, standard groups are compared with control group and values are represented as The mean±SEM; n=3;P<0.05;P<0.01 vs control.

Moreover, the degree of wound healing in the extracts treated group and standard drug was not significantly different. On Day 30 after burn injury, the degree of wound healing in animals in all extract-treated groups was significantly higher than that in the vehicle control group. The degree of wound healing did not differ significantly among the extract-treated groups.

DISCUSSION: The present study demonstrated the effect of the Centella asiatica and Elettaria cardamom extract on the wound healing process inflicted in the rats by the burn model. In burn model there is extensive loss of tissues and cells and this makes the healing process complicated. The groups treated with the extracts of Centella asiatica and Elettaria cardamom on the days 6, 12, 24 showed significant increase in the wound healing as compared to the control treated groups. On the day30 maximum amount of the healing was seen in all the groups, all extract-treated wounds appeared to heal better than the controls based on gross examination and degree of wound healing. The different chemical constituents in the extracts are responsible for the wound healing. Asiatic acid in the centella asiatica and linalool in the Elettaria cardamom is responsible for the healing process and matrix remodeling. The results of this study support the notion that Centella asiatica and Elettaria cardamom can promote wound healing by inhibiting inflammation, inducing collagen synthesis, promoting angiogenesis, inducing vasodilation, and reducing wound oxidative stress. In addition, Centella asiatica extracts have been shown to affect cellular growth and proliferation in injured tissues[23] In an ideal wound healing situation, new tissue growth replaces damaged tissue causing functional or cosmetic impairment. The wound healing activity of Centella asiatica extracts may be related to the growth factors such as endothelial growth factor, fibroblast growth factor and vascular endothelial growth factor. A further study on the effects of Centella asiatica extract on these growth factors is needed to clarify the mechanism of wound healing. Microcirculatory studies are also needed to investigate the anti-inflammatory activity of Centella asiatica and elletaria cardamom, which was seen early during the healing process[24].

CONCLUSION

The present study demonstrates the wound healing property of the Centella asiatica and Elettaria cardamom extracts on the burn wound model. Both the extracts are effective in complete wound healing and asiatic acid in centella asiatica and linalool in elettaria cardamom is the principle constituent for the activity of the drugs.

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