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EVALUATION OF WOUND HEALING ACTIVITY OF ETHANOLIC EXTRACT OF *LEPIDAGATHIS CRISTATA*. WILLD. (ACANTHACEAE) IN EXPERIMENTAL ANIMAL MODELS.

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ABSTRACT

Wound healing activity of ethanolic extract of *Lepidagathis cristata*. Willd. (Acanthaceae) was investigated by excision wound healing model in Wistar albino rats. Phytochemical screening was done to determine major constituents of ethanolic extract of *L. cristata*. Phytochemical study revealed that the presence of tannins, saponins, terpenoids, flavonoids and alkaloids. The wound healing effect was comparatively evaluated with simple ointment and standard drug nitrofurazone for a period of 18 days. Ethanolic extract of the herb showed significant reduction in wound area in comparison with control from 4th day ($p<0.01$) on wards and very high from 8th day to 18th day ($p<0.001$). The effect produced by the extract , in terms of wound contracting ability, wound closure, decrease in surface area of wound, and tissue regeneration at the wound site were significant in treated rats. The present study thus provides a scientific rationale for the traditional use of this plant in the management of the wound healing process.

KEYWORDS: *Lepidagathis cristata*, excision wound healing model, nitrofurazone, wound closure, tissue regeneration.

INTRODUCTION

Traditional medicine, play a significant element in the cultural heritage, still remains the main choice for a large majority of people for treating various diseases and ailments. More than 1000 medicinal plants (89.93%); 58 minerals, metals, or ores (5.24%) and 54 animal and marine products (4.86%) are used to control various forms of diseases like diabetes, cardiovascular disorders, hepatoprotective, antibacterial, antifungal and the wound healing.[1]

Wounds are defined as "skin defects caused by mechanical, thermal, electrical and chemical injuries, or by the presence of an underlying medical or physical disorder" by Thomas in1990.[2-10] The wounds are classified into the following three categories:

a) Chronic wounds are those that involve longer healing times ranging from months to years, for example pressure sores and leg ulcers. The chronic wounds fail to heal even though all efforts are undertaken to aid healing and become persistent or reoccur after a period of time.[11, 12] Commonly, it is known that if any wound does not heal within three months, it is considered as a chronic wound.

b) Acute wounds are defined as disruptions in the integrity of the skin and underlying tissues that progress through the healing process in a timely and uneventful manner.[13]

c) Post-operative wounds are intentional acute wounds.[5]

Wounds are the unpreventable events of life. It may be produced by physical, chemical, thermal, microbial or immunological damage to the tissue.[14] It results in the destruction of epithelium and also underlying connective tissue.[15,16] Current estimates indicate that worldwide nearly 6 million people suffer from chronic wounds.[17] Unhealed wounds always produce pain and inflammation at the wound site. Chronic wounds may even lead to multiple organ failure or death of the patient.[18] Wound healing process includes constant interactions between cell–cell and cell–matrix that proceed in to three overlapping phases viz. inflammatory phase(0–3days), cellular proliferation or proliferative phase (3–12 days) and remodeling phase (3–6 months).[19-21] Healing requires the synergistic efforts of various tissues and cell lineages.[22] It involves accumulation of platelets, clotting of blood, fibrin formation and an inflammatory response to injury, modification in the ground substances, angiogenesis and re-epithelialization. Disrupted wound healing places are firmly amalgamated by collagen.[23] The basic principle behind optimal wound healing is to minimize tissue damage and provide adequate tissue

perfusion and oxygenation, proper nutrition to tissue and moist wound healing environment to restore the anatomical continuity and function of the wound.[24] Wound healing process comprises coagulation, inflammation, proliferation, formation and accumulation of fibrous tissues, collagen deposition, epithelialization, contraction of wound with formation of granulation tissues, remodeling and maturation.[25]

Medicinal plants have been used since time immemorial for treatment of various ailments of skin and dermatological complaints like cuts, wounds and burns. Wound healing disorders leads to serious clinical problem and are associated with diseases such as diabetes, hypertension and obesity. In India, a popular system of Indian medicine Ayurveda was bestowed on lot6 of information on wound healing properties of medicinal plants.[26] Classic administration of wounds follows different therapeutic steps, starting from the sterile clothing and ending with the reestablishment of the natural structure and function.[27] The aim of these therapeutic measures is not only to speed up the healing process, but also to maintain the quality and aesthetic of healing. As described in the literature about 70% of wound healing drugs are of plant origin, 20% from metal and the remaining 10% from animal products.[27] These drugs are highly effective in controlling various types of wounds such as wounds &ulcers, sinusitis, abscess, sore syphilis, larvae in wounds, wounds sewage, inflammatory changes of wounds, cellulitis, purulative ulcer, cinder diabetes and fistula infrastructure anus. A recent research reported that there are about 450 plants species having wound healing properties.[25] These medicinal plants have been shown to possess wound healing activity in animal studies.[28, 29]

Lepidagathis cristata Willd. (Acanthaceae) (*L. cristata*) is a medicinal herb (Figure 1) and used as bitter tonic in fevers and used in pneumonia, flu, mouth infections.[30], eczema, psoriasis and other skin infections.[31] The ash of whole herb is applied externally on chronic wounds of pet animals.[32] The roots of the herb are used in stomachic and dyspepsia, leaves are used for fevers and the inflorescence ash is used for itchy affections of skin and burns.[33, 34] Leaf juice with copper sulphate is given during snakebite for gaining consciousness.[35]

Figure 1. *Lepidagathis cristata* plant habit.

The plant is a stiff herb and the branches procumbently arise from a hard central rootstock. Leaves are alternate, elliptic, serrate and usually lineolate. Flowers are sessile, capitate, the heads terminal or axillary densely crowed at the base of the plant, fruits glucose capsule.[36, 37] This medicinal herb has been exploited tremendously by common people in many ways for various curative purposes. It is necessary to evaluate the herb in a scientific base for its potential use of folk medicine for the treatment of infectious diseases.[38] Antibacterial studies[38,39], antifungal studies[40-42], pharmacognostical and phytochemical studies[43], analgesic and anti-inflammatory activities studies[44], hypoglycemic activity in alloxan induced diabetic rats of *L. cristata* have been documented[45], so far but wound healing activity on animal model of this herb seems to be lacking. Biological studies are very much essential to substantiate the therapeutic properties of medicinal herbs used in folk medicine on scientific bases.[46] Literature survey on *L. cristata* revealed that the therapeutic properties of this herb had not been established so far. Hence an attempt was made in the present study to evaluate wound healing activity of ethanolic extract of *Lepidagathis cristata* on albino rats.

MATERIALS AND METHODS

Collection and identification of plant material

Fresh plants *of L. cristata*, Willd. (Acanthaceae) were collected from Pachhaimalai Hills, Tiruchirappalli District. The taxonomic identities of the plant were confirmed by previously described.[36]

Extraction of plant material

The plant material was washed under running tap water, air dried in shade and then pulverized to obtain a course powder and then was subjected to solvent extraction by soxhlet apparatus. The extraction was carried out for 72 hours until the extract becomes colourless. Then the solvent was completely removed by evaporating in rotatory flask evaporator. The dried extract thus obtained was kept in desiccators and was used for further experiment. Percentage yield of ethanolic extract of *L. cristata* was found to be 1.95%w/w.[47]

Preliminary phytochemical screening

The phytochemical screening was done according to the references to the following chemical groups: saponins, tannins, terpenoids, alkaloids and flavonoids.[48]

Evaluation of wound healing activity

Experimental animal

The healthy Wistar albino rats of either sex and of approximately the same age, weighing about 220-225g were obtained from Department of Pharmacology, Periyar College of Pharmaceutical Science, Tiruchirappalli. The rats were kept under standard environmental conditions of temperature (37± 2°C) and humidity (35-60%) with 12:12 hour light/dark cycle in polypropylene cages. The animals were fed with standard pellet diet and water *ad libitum* throughout the

experimental period. Rats were acclimatized to laboratory conditions one week prior to start the experiment. Experiments were conducted in accordance with the internationally accepted principles of laboratory animal use and care. The study protocol was approved by the Institutional Animal Ethics committee (Reg.No:265/CPCSEA/2015-2016) for the purpose of control and supervision of animals (CPCSEA, New Delhi).

Ointment preparation

Table 1 shows the preparation of simple ointment. Table2depicts 5% (w/w) ethanolic extracts of *L. cristata* with molten mixture of white soft paraffin, cetosteryl alcohol, polysorbate 60, butylated hydroxyl anisole, glycerin and purified water.[49]

Table 1. Ingredients for the preparation of simple ointment

Ingredients	Quantity(g)
Polysorbate 60	5.0
White Soft Paraffin	25.0
Cetosteryl alcohol	4.0
Glycerin	12.0
Butylated hydroxyl anisole	0.02
Purified water	*q.*sto 100

Table 2. Ingredients for the preparation of extract of *L.cristata*

Ingredients	Quantity(g)
Ethanolic extracts of *L. cristata*	5.0
Polysorbate 60	5.0
White Soft Paraffin	25.0
Cetosteryl alcohol	4.0
Glycerin	12.0
Butylated hydroxyl anisole	0.02
Purified water	*q.*sto 100

Acute dermal toxicity study

The acute dermal toxicity study was carried out in adult albino rats of both sexes by "fix dose" method of OECD (Organization for Economic Co-operation and Development) Guideline No.434.[50] The prepared ethanolic extract of *L. cristata* was applied topically at a dose level of 2000mg /kg on the shaved part of albino rats for 14 days. The creams were applied with porous gauze dressing to make contact with the skin. The animals were observed for any changes in skin rashes or dermatitis or death.

Experimental design

The animals were divided into three groups of six rats each

Group I served as control (simple ointment) (Table 1)

Group II served as standard (Nitrofurazone 0.2% w/w) treated with ointment topically

Group III served as test treated with *L.cristata* ethanol extract ointment (5%w/w) (Table 2).

Excision wound model

All animals in each group were anaesthetized by the open mask method with anaesthetic ether before wound creation. The rats were depilated on the dorsal thoracic region.[51] An excision wound was inflicted by cutting away a 500mm^2 full thickness of skin from depilated areas. Haemostasis was achieved by blotting the wound with cotton swab soaked in normal saline, the wound was left undressed to open environment. Then the drugs were applied topically once in daily till the wound was completely healed. This model was used to monitor wound contraction and wound closure time.

Measurement of wound contraction

Wound contraction was calculated as percent reduction in wound area. The progressive changes in wound area were monitored planimetrically by tracing the wound margin on graph paper on every alternate day.

Wound contraction (%) was calculated using the following formula[52]:

Wound contraction (%) = $[(WD0-WDt)/WD_0]$ 100

Where: WD0=the wound diameter on day zero; WDt=the wound diameter on day t.

Statistical analysis

Results are expressed as mean ± SE. The differences between experimental groups were compared by student t' test (control vs. treatment) and was considered statistically highly significant when $p<0.01$ and significant when $p<0.001$.

RESULTS AND DISCUSSION

The phytochemical screening of the ethanolic extracts *L.cristata* revealed the presence of tannins, saponins, steroids, terpenoids, flavonoids and alkaloids. It was reported that the flavonoids and saponins possess significant wound healing activity.[53] Rats of either sex applied with the extracts up to a dose of 2000 mg/kg for fourteen days did not produced any signs of toxicity and mortality. The animals were observed for tremors, convulsions, salivation, diarrhea, lethargy, sleep behavioral and clinical abnormalities, gross lesions, body weight changes and coma from day 1 to 14 days. The animals were found to be physically active and they were consuming food and water in a regular way. The values presented in the table 3 represent wound area (mm^2) and percentage of wound contraction at 2, 4, 6,8,10,12,14,16, and 18 days for control (simple ointment), standard ointment (nitrofurazone 0.2%) and ethanol extract of *L.cristata*. A significant ($p<0.01$) wound healing property was observed in group III than group II & I on 8th, 10th and 12th days. The percentage of closer of wound was found to be significant ($p< 0.001$) where the animals treated with standard and ethanol plant extract showed 93% & 92% on 14th day and 98% & 96% on 16th day respectively which is very closer to that of standard nitrofurazone. The complete contraction of wound (100%) was observed on 18th day in group III& II where as group I showed 68% only (Table 3, Fig 2 & Fig 3).

Table 3. Effect of ethanolic extract of *L. cristata*, nitrofurazone and simple ointment on % of wound contraction of excision wound models.

Post wounding days	Control (simple ointment)		Standard ointment (Nitrofurazone 0.2%w/w)		Ethanol extract of *L. cristata* (5%w/w)	
	Wound area(mm^2)	% of wound contraction	Wound area(mm^2)	% of wound contraction	Wound area(mm^2)	% of wound contraction
Day 0	530±33.6	0	516±36.8	0	514±21.0	0
Day 2	509±18.6	4	458±36.8	11	372±18.8	27
Day 4	465±13.8	12	318±12.6*	38	312±19.9*	39
Day 6	424± 30.1	20	270±14.7*	48	245±15.3*	52
Day 8	389±14.8	27	193±11.4**	63	162±12.5**	68
Day 10	345±23.6	35	110±8.6**	77	95±9.6**	81
Day 12	269±14.3	49	79±6.3**	85	66±7.4**	87
Day 14	215±11.3	59	36±1.6**	93	37±3.5**	92
Day 16	189±14.3	64	10±1.9**	98	19±0.8**	96
Day 18	171±15.1	68	0.0**	100	0.0**	100

Values are means ± S.E of 6 animals in each group. * Significant differences at p<0.01 when compared to control. **Significant differences at p<0.001 when compared to control by student t-test.

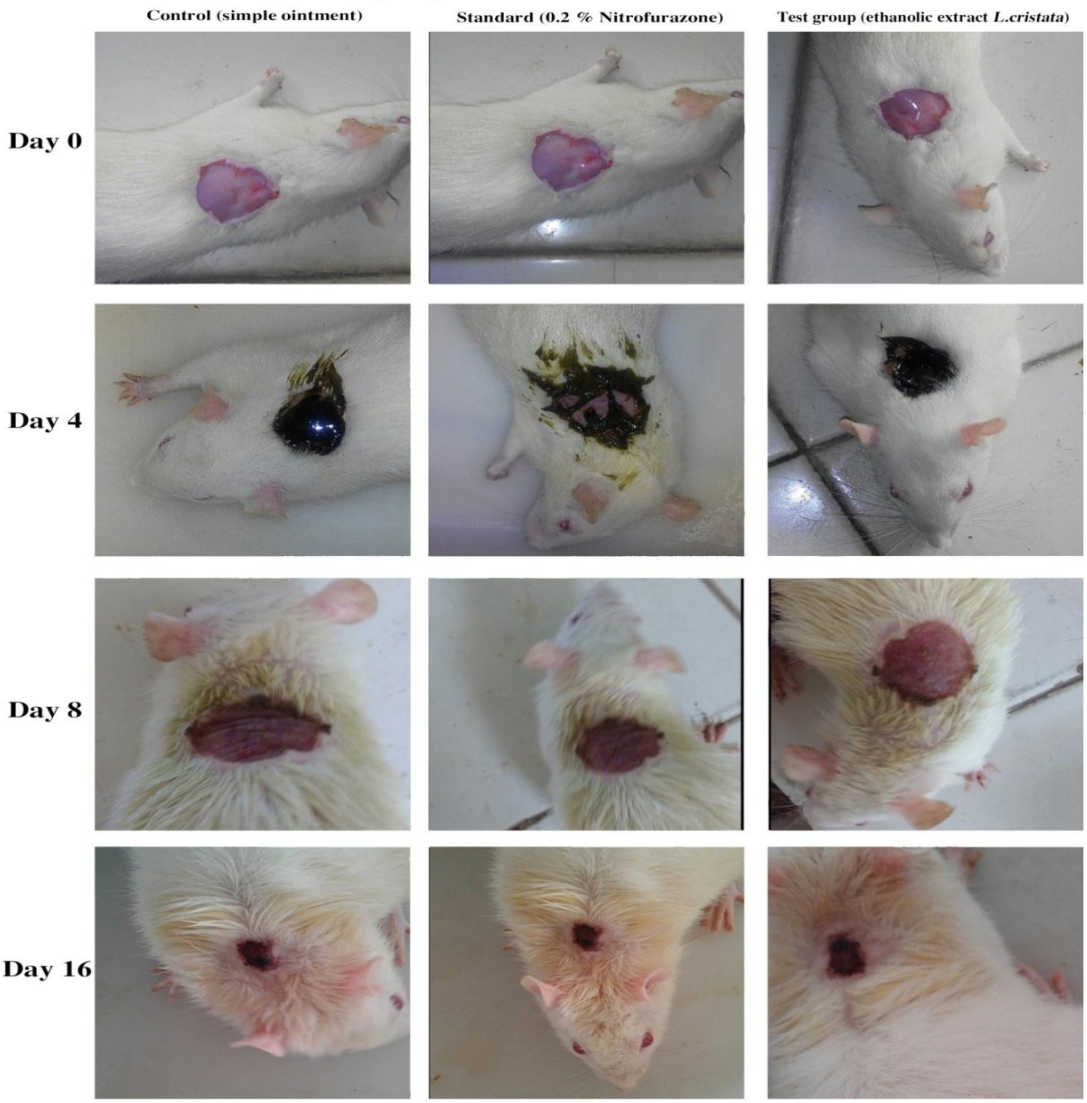

Figure 2. Comparison of wound site by excision wound model in control, standard and test group (ethanolic extract of *L. cristata*)

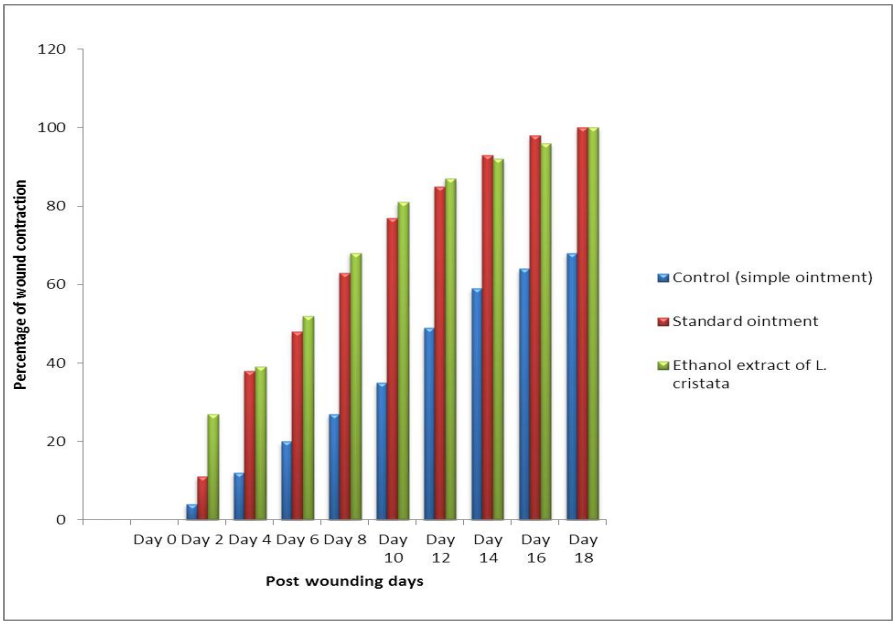

Figure 3. Effect of ethnolic extract of *L.cristata* on wound healing process in rats.

Medicinal plants have great potentials and have been shown to be very beneficial in wound care, promoting the rate of wound healing with minimal pain, discomfort and scarring to the patient. Some of these plants owe their effects to direct effect on the wound healing process. The present investigation showed that the ethanolic extract of *L.cristata* has significant wound healing property. This plant was preferred for the evaluation of wound healing activity because of its non-toxicity and absence of unwanted side effects. The active ingredients present in this plant are anticipated to interfere with one or more phases of the wound healing process in a positive manner in proper sequence and at the right time frame to show improved efficacy. These secondary metabolites in plant extracts that could bind to cellular receptors at wound site to initiate modulation of wound healing process was recently reviewed.[54] Terpenoids are known to boost wound healing process due to their astringent and antimicrobial properties which seems to be responsible for wound contraction and epithelialization.[55] In the same way other bioactive compounds like flavonoids[56], anthocyanins[57], phenolics[58] , caffeic acid[59], chlorogenic acid[60], ferulic acid[61], water soluble alkaloids like indole derivatives in *Adhatoda vasica*[62] and *Adhatoda zeylanica*[63] are reported to promote wound healing process appreciably. Topical application of ethanol extract of *L.cristata* Willd. (Acanthaceae) to an infected wound not only reduces the rise of further infection but also improves the healing activity. It also found to improve to improve the different phases of wound repair, including collagen synthesis and maturation, wound contraction and epithelialization.

CONCLUSION

This study shows that ethanolic extract of *L.cristata* has potential wound healing effect when formulated as ointment and suggests further study in this herb.

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