

Evaluation of the Wound Healing and Anti-Inflammatory Activity of Whole Plant of *Luffa cylindrica* (Linn). in Rats

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Summary

The aim of the present investigation was to evaluate the wound healing and anti-inflammatory activity of chloroform extract of whole plant of *Luffa cylindrica* (Linn). The chloroform extract were prepared from the whole plant of *Luffa cylindrica* (Linn) by hot continuous percolation method in Soxhlet apparatus. The wound healing activity was evaluated by excision wound model. A maximum wound healing activity was found in chloroform extract *Luffa cylindrica* in comparison with standard. The anti-inflammatory activity was evaluated using carrageenan-induced rat paw edema method in rats. The effects of the administration of reference standard (Ibuprofen at the dose 10mg/kg) were also evaluated. The chloroform extracts of *Luffa cylindrica* at the dose level of 25 and 50 mg/kg, p.o. were tested. Treatment with chloroform extract of *Luffa cylindrica* at the dose of 50 mg/kg had showed significant ($p < 0.01$) inhibition of carrageenan induced rat paw edema than that of rats were received 25mg/kg. The results obtained indicate that chloroform extracts of *Luffa cylindrica* has wound healing and anti-inflammatory activities that supports the folk medicinal use of the plant.

Keywords: *Luffa cylindrica*, wound healing activity, anti-inflammatory activity.

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Introduction

A wound may be defined as a break in the epithelial integrity of the skin or may also be defined as a loss or breaking of cellular and anatomic or functional continuity of living tissue¹. Wound healing studies are mainly aim to detect various means and factor influencing healing process, so they could be either used or avoid in clinical practice to favourably alter the healing process².

In India, medicines based on herbal origin have been the basis of treatment and cure for various diseases³. Moreover, Indian folk medicine comprises numerous prescriptions for therapeutic purposes such as healing of wounds, inflammation, skin infections, leprosy, diarrhoea, scabies, venereal disease, ulcers, snake bite, etc⁴. More than 80% of the world's population still depends upon traditional medicines for various skin diseases⁵. Herbal medicines in wound management involve disinfection, debridement and providing a moist environment to encourage the establishment of the suitable environment for natural healing process⁶.

Luffa cylindrica (Linn). Commonly called sponge gourds. Plant belongs to the curcubitaceae family. The fruits, which also have a network of fibres surrounding a large number of flat blackish seeds. It is reported to have originated from India⁷. *Luffa cylindrica* has been reported to possess both medicinal and nutritional properties. Its seeds have been used in the treatment of asthma, sinusitis and fever⁸. It also reported that abortifacient proteins⁹ such as luffaculin which possess ribosome-inhibiting properties on the replication of HIV infected lymphocyte and phagocyte cells explain its potential as a therapeutic agent for AIDS¹⁰. It has been reported that juice extracted from the stem has been used in the treatment of respiratory disorders and the seed has emetic action. The aim of the present investigation was to wound healing and anti-inflammatory activity of chloroform extract of whole plant of *Luffa cylindrica* (Linn) with respective animal models.

Materials and Methods

Collection and identification of plant materials

The whole plant of *Luffa cylindrica* (Linn) was collected from Tirunelveli District, Tamil Nadu, and India. The whole plant were identified, confirmed and authenticated by comparing with an Authentic Specimen by a Botanist Dr.P. JAYARAMAN, Ph.D., Plant Anatomy Research centre (PARC), West Tambaram, Chennai-45. The whole plant were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

Preparation of Extracts

The powdered plant material was successively extracted with chloroform by hot continuous percolation method in Soxhlet apparatus¹¹ for 24h. Then the extract was concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

Experimental Animals

Healthy male Wistar rats weighing 120-150g were procured from National Institute of Nutrition, Hyderabad, India, and the animal experiments were performed in accordance with legislation on welfare (CPCSEA). The animals were fed with standard laboratory diet and were provided clean drinking water *ad libitum*. The animals were acclimatized to laboratory conditions for a week prior to the initiation of the experiment. Twelve hours before the start of the experiment.

Wound Healing Activity

Excision Wound¹²

Animals were anaesthetized prior to and during creation of wounds, with ether. The dorsal fur of the animals was shaved with an electrical clipper and the anticipated area of the wound to be created was outlined on the back of the animals with methylene blue using a circular stainless steel stencil. An excision wound was inflicted by cutting away 500mm² full thicknesses along the marking on the depilated back 5cm away from the ears.

Experimental Design

The animals were randomly divided into four groups of six each as follows: Group I rats were treated with simple ointment base (control). Group II rats treated with the reference standard 2% w/w Mupirocin, Group III and IV rats were treated with 5% w/w & 10% w/w of extract ointment respectively. The extract ointments at a quantity of 0.5g were applied once daily to treat different groups of animals. Wound closure rate was assessed by tracing the wound on

every alternate days of post wounding using transparency paper and a permanent marker. The wound areas recorded were measured using graph paper. The wound was left undressed to the open environment. The day of eschar falling off, after wounding, without any residual raw wound was considered as the time until complete epithelialization.

Anti-inflammatory activity

Carrageenan induced rat paw oedema¹³

Twenty four rats were divided into 4 groups of 6 rats each for various treatments as shown in Table. Subsequently 1 hr after treatment, 0.1ml of 1% carrageenan was injected subcutaneously into the planter region of right hind paw to induce oedema. The paw volume was measured initially and at 1,2,3 and 4 hr after carrageenan injection using Plethysmometer.

Statistical Analysis

Results of all the above estimations have been indicated in terms of mean \pm SEM. Difference between the groups was statistically determined by analysis of variance (ANOVA) with Dunnett's test multiple comparisons test using SPSS package. The level of significance was set at $P < 0.05$.

Results and Discussion

1. Wound Healing Activity:

Wound healing is the primary response to tissue injury with different phases like contraction, granulation, epithelisation and collagenation which is mainly achieved by connective tissue matrix synthesis^{14, 15}. Wound healing is a complex and dynamic process of restoring cellular structures and tissue layers in damaged tissue as closely as possible to its normal state. Table 1 shows the effects of the chloroform extract of *Luffa cylindrica* on wound-healing activity in rats in the excision wound model, *Luffa cylindrica* treated animals showed a significant reduction in the wound area and period of epithelization. Significant wound-healing activity was observed in animals treated with the chloroform extract of *Luffa cylindrica* compared to the control treated groups.

Table 1: Effects of the chloroform extract of *Luffa cylindrica* on wound-healing activity in rats in the excision wound model

Post wounding days	Wound area in mm ²			
	Control (Simple ointment)	Mupirocin (2%w/w)	Extract (5%w/w)	Extract (10% w/w)
1	512 \pm 5.35	505 \pm 6.55	501.5 \pm 4.92	510.3 \pm 7.60
2	506.8 \pm 4.70	482.3 \pm 3.9 ^a (4.50)	492.3 \pm 6.98 (1.83)	486 \pm 6.57 (4.76)
4	503 \pm 3.76	398.5 \pm 9.58 ^{ab} (21.09)	480.5 \pm 5.56 (4.19)	473.8 \pm 4.92 ^a (7.15)

8	490.8 ± 5.20	199.3 ± 4.82 ^{ab} (60.53)	463.5 ± 5.56 ^a (7.58)	358.8 ± 6.25 ^{ab} (29.69)
12	385.5 ± 5.95	49.25 ± 3.2 ^{ab} (90.25)	339.8 ± 5.68 ^{ab} (32.24)	202 ± 3.29 ^{ab} (60.42)
14	309 ± 4.74	(100)	253.8 ± 4.64 ^{ab} (49.39)	134 ± 5.21 ^{ab} (73.74)
16	262.5 ± 3.71	(100)	125 ± 2.48 ^{ab} (75.08)	45.75 ± 3.20 ^{ab} (91.03)
18	201.3 ± 4.99	(100)	37.5 ± 3.28 ^{ab} (92.52)	(100)
21	144.5 ± 4.98	(100)	(100)	(100)

* All the values are expressed as mean ± SEM (n=6).

Statistical Significance was calculated by ANOVA followed by post hoc Dunnett's using SPSS package (a p<0.05; b p<0.01).

2. Anti-inflammatory Activity

Carrageenan-induced paw edema and cotton pellet granuloma formation in rats reflect the edematous stages during acute and chronic inflammation^{16, 17}. In the present study the chloroform extract of *Luffa cylindrica* (doses at 50mg/kg and 25mg/kg) was evaluated for anti-inflammatory activity using carrageenan-induced rat paw edema and the data was compared with that of control (Table 2). Treatment with chloroform extract of *Luffa cylindrica* (25mg/kg and 50mg/kg) had showed significant inhibition of carrageenan induced rat paw edema. Maximum inhibition was observed at 50 mg/kg dose as compared to the control. It was observed that the chloroform extract of *Luffa cylindrica* (50 mg/kg, p.o.) exhibits maximum anti inflammatory activity against carrageenan induced hind paw edema.

Table 2: Effects of the various extracts of *Luffa cylindrica* on extracts on rat paw edema induced by carrageenan

Time (in hrs)	Paw volume in ml			
	Control	Ibuprofen (10mg/kg)	Extract (25mg/kg)	Extract (50mg/kg)
0	0.24 ± 0.01	0.22 ± 0.035	0.25 ± 0.012	0.26 ± 0.01
1	0.28 ± 0.023	0.25 ± 0.04	0.28 ± 0.02	0.25 ± 0.014
2	0.31 ± 0.031	0.28 ± 0.03	0.3 ± 0.012	0.28 ± 0.014
3	0.37 ± 0.04	0.22 ± 0.03 ^a	0.29 ± 0.024	0.27 ± 0.03
4	0.38 ± 0.04	0.2 ± 0.014 ^{ab}	0.27 ± 0.012	0.23 ± 0.03 ^a
5	0.34 ± 0.03	0.18 ± 0.02 ^{ab}	0.22 ± 0.015 ^a	0.2 ± 0.03 ^{ab}
6	0.3 ± 0.03	0.15 ± 0.003 ^{ab}	0.20 ± 0.014 ^a	0.18 ± 0.014 ^{ab}

All values are expressed as Mean ± SEM; n=6.

Statistical Significance was calculated by ANOVA followed by post hoc Dunnett's using SPSS package (a p<0.05; b p<0.01).

Conclusion

From the present study, it is concluded that the chloroform extract of *Luffa cylindrica* had showed the significant wound healing and anti-inflammatory activity. As infections being a major cause of morbidity and mortality in wound patients, this plant extract may prevent infection that leads to high risk of sepsis, and thereby prevents the prolongation of inflammatory phase. Further study on the fractionation of active components and the mutual effect of these plant extract machinery on infecting microbial species may provide a better understanding of the infection management in the process of wound healing.

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