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WOUND HEALING AND ANTIBACTERIAL ACTIVITIES OF THE CHLOROFORM EXTRACT OF *GLINUS LOTOIDES LINN.* IN ALBINO RATS

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ABSTRACT

Wound healing is a process which is fundamentally a connecting tissue response. Wound healing comprise of different phase such as contraction, epithelization, granulation and collagenation. Microbes easily enters into the wounds and causes infection.The present study has proved that the wound healing potential and antibacterial activity of chloroform extract obtained from *Glinus lotoides Linn.* showed significant effect. The animals administrated with the chloroform extract *Glinus lotoides Linn.* (CEGL) showed a high tensile strength and antibacterial activity. Flavanoides, glycosides may be attributed to the wound healing activity.

KEY WORDS: Wound healing, Glinus lotoides Linn., Excision wound, Incision wound model.

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INTRODUCTION

India has rich source of flora and fauna [1]. Herbs have always been the principal form of medicine in India and presently they are becoming popular throughout the world. Plants are less toxic more natural and accessible than manufactured drug [2]. The objective of this investigation was to ascertain the scientific basis for the use of the chloroform extract of *Glinus lotoides Linn.* for the treatment of wound. *Glinus lotoides Linn.* is an annual or short living perennial prostrate herb, the seeds of which are traditionally used in Ethiopia as anthelminthis[3,4] and in India and Pakistan as antifungal and antitumor [5,6]. The aim of wound care is to promote wound healing in the shortest time possible with minimal pain, discomfort and scarring to the patient and must occur in a physiologic environment conducive to tissue repair and regeneration[7]. Bacteria directly invade wounds producing inflammation and fluid exudation which interferes with the healing process. The bacteria toxins cause tissue damage and delay fibroplasias as well as collagen synthesis[8]. However very little information has been published on this plant and there is no scientifically proven data to show whether the *Glinus lotoides Linn.* chloroform extract has wound healing activity or not. Therefore we have undertaken the present study to explore the effects of the above extract on wound healing.

MATERIALS AND METHODS

The present project was carried out in the department of Biochemistry at V.V.Vanniaperumal College for Women, Virudhunagar, Tamilnadu, India. The preliminary work (extraction, Phytochemical studies) and antibacterial activity was done in V.V.Vanniaperumal College For Women, Virudhunagar, Tamilnadu,India. The wound healing activity of drug on rat study was carried out in the Sangaralinkam Bhuvaneswari college of Pharmacy, Annaikuttum, Tamilnadu, India. The *Glinus Lotoides Linn* powder was collected from Sri Harshini Herbals at Madurai, Tamilnadu, India.

Extraction Procedure

The *Glinus lotoides* powder was extracted with petroleum ether by cooled extraction method for 72 hours. Petroleum ether extract was filtered and evaporated under reduced pressure. The extracted plant material was then air-dried, again treated with chloroform for 72 hours. The chloroform extract was filtered and evaporated under reduced pressure. The extracted plant material was then air dried again treated with ethy1acetate for 72 hours[9]. These three samples were taken to carry out the phytochemical studies to detect the presence of phytoconstituents[10].

Ointment Formulation (British pharmacopoeia, 1993)

A control ointment base was formulated without any drug content. Two creams were formulated by using 5% and 10% extract (5gm and 10 gm of dried chloroform extract of *Glinus lotoides Linn.* were incorporated in 100gm of cream base). The standard drug for screening wound healing activity is Povidone iodine ointment (5%w/w) which was bought commercially.

Animal Model

Albino rats (150-250gm) of either sex were procured from animal house of Sangaralinkam Bhuvaneswari College of Pharmacy (Regd. No.622/02/C/CPCSEA) used for the present study. The Albino rats were divided into four groups of six rats. Groups I rats were treated with simple ointment base (control). Group II rats were treated with a reference standard Povidone iodine ointment. Group III and IV rats were treated with 5% and 10% ointment respectively.

Excision Wound Model

This model was employed to study the rate of wound contraction and epithelization. A round seal of 2.5 cm in diameter was impressed on the dorsal thoracic central region 5 cm away from the ears. The entire thickness of the skin from demarked area was excised to get a wound measuring around 500mm^2. Animals were subjected to the treatment from '0' day till the wound completely healed or up to 21st post wounding day, which ever was earlier. The observations of percentage wound contraction were made on 2nd, 6th, 10th and 14th post wounding days. Number of days required for falling of the Escher without any residual raw wound gave the period of epithelization [11].

Incision Wound Model: [12, 13]

Two 5cm long Para vertebral incision were made through the entire thickness of the skin on either side of the vertebral column of the rat. Wounds were closed with interrupted sutures 1 cm apart. The creams of *Glinus lotoides Linn.,* Povidone iodine cream and control cream were applied to the wound twice daily till complete healing. The sutures were removed on the 9th day and tensile strength was measured with a Tensiometer.

Tensile Strength

The tensile strength of a wound represents the degree of wound healing. Usually wound-healing agents promote a gain in tensile strength. The sutures were removed on the 9th day after wounding and the tensile strength of removed tissue was measured on the 10th day. The formulated cream and standard drug were applied twice daily. The skin breaking strength of the 10th day old wounds was measured[14].

Antibacterial Activity

The antibacterial activities of all the newly synthesized compounds were determined by well plate method in Muller Hinton agar. The antibacterial activity of the test compounds were assayed against *Bacillus subtilis, Staphylococcus aureus, E.coli* and *Proteus vulgaris*. The overnight cultures were grown at 37^{0}c. Bacterial suspensions of 10X10^8 colony-forming units (CFU) per ml. the extracts were loaded in the wells of micro plates at concentrations of 0.5g/ml and incubated at 37^{0}c for 24 hours, the diameters of the zone of inhibition(cm) surrounding each of the wells were recorded. Tetracyclin and Streptomycin (0.2g/ml) were used as standard antibiotic.

Statistical Analysis

The results were expressed as mean ± SEM and analyzed by statistically using one way ANOVA followed by Dunnett's test.

RESULTS AND DISCUSSION

The preliminary phytoconstituents of different extracts were shown in the Table 1. Chloroform extract of Glinus lotoides Linn. showed the presence of alkaloids, sterols, glycosides, flavanoids, phenols and triterpenoids .It was also reported that the flavanoids, glycosides may be attributed to the wound healing activity [15]. Among these extract the two main constituents were present in the chloroform extract, so we choose the chloroform extract for our wound healing study.

Table 1
Preliminary Phytoconstituents of different extract

S.No	Phytoconstituents	Petroleum ether	Chloroform	Ethyl acetate
1	Protein	-	+	+
2	Alkaloids	+	-	-
3	Carbohydrates	-	-	-
4	Sterols	+	+	+
5	Glycosides	-	+	-
6	Flavanoids	-	+	-
7	flavanones	-	-	-
8	Phenols	-	+	+
9	Quinones	-	-	-
10	Phyto sterols	+	+	-
11	Triterpenoids	-	-	+
12	Oil	+	-	-
13	Saponines	-	-	-

+ = Presence , - = Absence

The Progress of the wound healing induced by chloroform extract ointment (5% and 10%w/w) treated groups, simple ointment (control) treated group and povidone iodone ointment (standard drug) treated group were shown in Table 2. Out of the two test extract ointment, (10%w/w) extract appears to be the best in promoting the wound healing in terms of wound contraction and epithelization period. However on 12th post wounding day Group I animals showed 48.5% of healing (which may be due to self immunity of the animals) where as povidone – iodine treated animals showed 83±1.30 healing. The extract treated group showed 144±2.16 and 91±2.905 healing at 5% and 10% respectively. epithelization was found to be enhanced significantly by chloroform extract of CEGL 5%w/w and 10%w/w oinment treated groups as evidence by shorter period required for eschar dropping (18.88± 0.2600 and 17.88±0.2909) as compared to control (21.80±0.222) and it also closely matching to the standard oinment. Similar results were obtained from the *Darvhi ghrita* showed 187.6±7.23 of wound healing on 12th post wounding day[16].

Table-2
Effect of Topical Application of Glinus lotoides Linn. on Excision wound model (Wound area mm^2)

Parameter	2nd day	4th day	6th day	8th day	10th day	12th day	14th day	16th day	18th day	20th day
Group I Control	511± 1.2905	470± 2.943	432± 2.38	393± 2.38	349± 1.290	274± 2.217	210± 2.6197	190± 1.7075	172± 2.905	145± 1.2905
Group II Standard	480± 2.16*	358± 3.10***	273± 2.629*	219± 1.30***	126± 2.59****	83± 1.83****	44± 1.83****	19± 1.30****	8± 0.95****	0
Group III CEGL 5% w/w Ointment Treated	485± 2.5$^{N.S}$	382± 1.25**	318± 2.76$^{N.S}$	280± 3.162*	206± 2.30****	144± 2.16****	96± 1.83****	54± 1.81****	17± 1.70****	0
Group IV CEGL 10% w/w Ointment treated	479± 1.707*	358± 3.10***	274± 5.057*	226± 1.92**	137± 1.30****	91± 2.905***	51± 1.29****	27± 1.23****	11± 1.29****	0

Values are expressed as mean ±SEM, n=6*p<0.05**p<0.02 ***p<0.01 ****p<0.001 vs normal control group. One way ANOVA followed by Dunnett's test

Table 3
Period of epithelization

Parameter	Period of epithelization (in days)
Group I (Control)	21.80 ± 0.222
Group II (Standard)	17.90±0.1*
Group III (CEGL 5% w/w Ointment treated)	18.88±0.2600*
Group IV (CEGL 10% w/w Ointment treated)	17.83±0.2909*

Values are expressed as mean ±SEM, n= 6*p< 0.01 vs normal control group. One way ANOVA followed by Dunnett's test.

In the incision model, the tensile strength of standard, 5% and 10% extract treated group was found to be 262g, 218 g and 257g respectively on the 10th post wounding day shown in Table 4. The entire drugs treated group was much greater than that of the control group. The tensile strength of the chloroform extract oinment treated group was closely matching to the standard Povidone –iodine oinment. Similar results were obtained from the ethanolic extract of the leaves of *Tagets erecta linn* showed 193.33g on 10th post wounding day[17]. The increased tensile strength of treated wounds may be increased collagen formation / unit area and stabilization of the fibers[18, 19].

Table 4
Effect of Topical application of Glinus lotoides Linn. on Incision wound model

Parameter	Tensile strength
Group I (Control)	146±2.5
Group II (standard)	262±2.5*
Group III (CEGL 5% w/w Ointment treated)	218±1.7078*
Group IV (CEGL 10% w/w Ointment treated)	257±1.2909*

Values are expressed as mean ±SEM n= 6*p< 0.01 vs normal control group. One way ANOVA followed by Dunnett's test.

Lipid peroxidation is an important process in several types of injuries like burns, infected wounds, skin, ulcers etc. Flavanoids are known to reduce lipid peroxidation not only by preventing or slowing onset of cell necrosis but also by improving vascularity [15] . Use of single model is inadequate and there is no reference standard which can collectively represent the various components of wound healing influence another. Hence our study we have two models to assess the effect of *Glinus lotoides Linn.* on various phases of wound healing. Wounds are known to be easy portals for infections and provides a suitable medium for the proliferation of microbial organisms. Wound infection has been identified as one of the most important factors that delays wound repair processes and outcome[20]. The antibacterial activity of various extracts of *Glinus lotoides Linn.w*as shown in Table 5.

Table 5
Antibacterial screening of various extracts of Glinus lotoides Linn. Against bacteria (Zone in cm)

Bacteria	Antiobiotic		Extraction		
	Streptomycin	Tetracyclin	Petroleum ether	Chloroform	Ethyl acetate
E.coli	0.5	1.0	0	0.4	0.2
Bacillus subtilis	0.6	1.1	0	0.4	0.3
Proteus vulgaris	1.1	1.3	0	0.3	0.2
Staphylococcus aureus	1.2	0.7	0	0.3	0.3

The chloroform extract of *Glinus lotoides Linn.* showed the best activity against *Bacillus subtilis, Staphylococcus aureus .* The inhibitory effect of CEGL was compared with the antibiotics tetracycline and streptomycin. The chloroform extract was closely matching to the antibiotics. Flavanoids, glycosides are known to promote wound healing process mainly by their astringent and antimicrobial property.

CONCLUSION

The results of the study showed that the chloroform extract of *Glinus Lotoides Linn.* possesses a definite pro-healing action and antibacterial activity. Flavanoids, Glycosides may be attributed to the wound healing activity.

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