



WOUND HEALING ACTIVITY OF *CALOTROPIS GIGANTEA* LEAVES IN ALBINO WISTAR RATS

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ABSTRACT

Calotropis gigantea Linn. (Asclepiadaceae) a widely growing plant has been reported to possess number of medicinal properties. It has been reported as a traditional folk medicine for a variety of alignments. The plant *C. gigantea* is also used in some parts of India for wound healing in combination with other plants. However there are no scientific reports on wound healing activity of the plant *C.gigantea*. The purpose of the present study was to evaluate scientifically the wound healing activity of Petroleum ether, Benzene, Chloroform, Methanol and Aqueous extract of leaves of the plant *C.gigantea* by excision and incision wound healing models in rats. Wistar albino rats of either sex weighing between 160 and 200 g were topically treated with extracts was applied once daily in excision wound model. *C.gigantea leaves* extracts were given orally at a dose of 200mg/kg and 180 mg/kg (methanol extract) in incision wound healing model. Rats of standard groups were treated with 5% Povidone iodine ointment topically. The percentage wound closure; epithelization time, hydroxyproline content and scar area on complete epithelization were measured. Topical application of methanolic extract of *C.gigantea* leaves in excision wound model increased significantly the percentage of wound contraction by 12th day, i.e. 70.11±0.54 compared with control 45.09±0.53. Scar area and epithelization time were decreased from 21.56 to 12.56 days when compared with control. In incision wound model breaking strength of wounds and hydroxyproline was increased significantly from 125.48±0.78 in control up to 321±0.99 with methanolic extract. Hence, the methanolic extract of *C.gigantea* leaves accelerated wound healing activity in rats and thus supports its traditional use.

Keywords: *Calotropis gigantea*, Wound healing activity, Excision wound model and Incision wound model

INTRODUCTION

Calotropis gigantea Linn. (Asclepiadaceae) a widely growing plant has been reported to possess number of medicinal properties¹. In the traditional system of medicine the roots and barks of *C. gigantea* are used as anticancer², anti-fertility³, antidote for snakebite, antiscabetic⁴, cardiovascular diseases⁵ and various skin diseases.

Leaves are used in asthma, skin diseases like eczema. Juice is used in leprosy, syphilis and idiopathic ulceration. Traditionally roots and barks of *C.gigantea* are used for all kinds of fits, epilepsy,

convulsions in children's and paralysis complaints⁶. The plant is reported to possess free radical scavenging⁷ and anti-diarrhoeal activity against castor oil induced diarrhea⁸. Flavonoids⁹,¹⁰, triterpenoids¹¹, volatile long chain fatty acids¹², glycosides and proteases¹³ have been isolated from the various parts of the plant *C. gigantea*. It is also used in some parts of India in wound healing in combination with other plants¹⁴.

The present study was carried out to determine effect of various extract of *C.gigantea* leaves on wound healing activity by excision and incision wound healing models using albino Wister rats of either sex.

EXPERIMENTAL

Plant: The fresh matured leaves of *C.gigantea* were collected locally from the suburban out fields of Bangalore, province of India. The identity of *C.gigantea* was authenticated by Dr.Siddamallayya from Regional Research Institute, Bangalore, on the basis of taxonomical characters following routine pharmacognostical studies, including organoleptic macroscopic tests and herbarium specimen was deposited in the department of herbarium. The leaves were air dried under shade and further subjected to extraction.

Preparation of extract¹⁵: The collected *C.gigantea* leaves were air dried under shade at room temperature and milled to a coarse powder. The obtained dried powder was subjected to successive soxhlet extraction with Pet.Ether (40-60⁰C), benzene, chloroform, methanol and finally the fresh drug was macerated with chloroform water. The powdered leaf material was packed in a tumble made of Whatmann's filter paper. It was subjected to extract with various non-polar to polar solvents for 40 cycles each. Each time before extracting with the next solvent the powdered material was air dried in hot air oven below 50⁰ C. The extract thus obtained was concentrated to dryness in a flash evaporator under reduced pressure and controlled temperature. The obtained residues were yellowish brown to dark brown colour with thick and sticky paste. The extract was stored in refrigerator (2-8⁰ C) and reconstituted uniformly in water for injection in the presence of tween 80 just before administration to animals orally using an intragastric feeding tube.

Animals: Adult rats of Wistar strain of either sex weighing 160-200 g and 10-12 weeks old were obtained from National Institute of Mental Health and Neuro Sciences. The study was approved by the Institutional Ethics Committee for animal experimentation. They were fed commercial pellet diet and water *ad libitum*. The diet approximately contained: carbohydrate (55%), fat (5%), protein (24%), fiber (4%), calcium (0.6%), phosphorous (0.3%), moisture (10%) and ash (9%). Before treatment allocation and randomization rats were acclimatized to the laboratory conditions for a week. Animals were housed in polypropylene cages (38X23X10cm) with not more than four animals per cage under standard laboratory conditions (25⁰ C ± 2⁰ C), relative humidity 55 ± 10%, alternating 10 h dark/14h light photoperiod. Approval from the institutional animal ethical committee for the usage of animals in the experiments was obtained and conducted in accordance to the Indian national

science academy guidelines for the use and care of experimental animals.

Acute toxicity studies: The acute toxicity studies were carried out according to OECD guidelines – 425. Rats of either sex (three females and three males, weight: 25–35 g, age: 6–8 weeks) received different extracts of *C.gigantea* leaves starting at 2 g/kg orally by gavage. The animals were observed for toxic symptoms continuously for the first 4 h after dosing. Finally, the number of survivors was noted after 24 h and these animals were then maintained for further 13 days with observations made daily¹⁶.

In vivo wound healing activity

Excision wound model:

The animals were divided into 7 groups of six rats each (Table1, Fig 1):

Group I: Served as control,

Group II: Served as standard treated with 5% Povidone iodine ointment topically

Group III: Animals received pet ether extract of *C.gigantea* 200 mg/kg, topically

Group IV: Animals received benzene extract of *C.gigantea* 200 mg/kg, topically

Group V: Animals received chloroform extract of *C.gigantea* 200 mg/kg, topically

Group VI: Animals received methanol extract of *C.gigantea* 180 mg/kg, topically

Group VII: Animals received aqueous extract of *C.gigantea* 200 mg/kg, topically

A standard uniform 2 cm diameter of wound was formed with the aid of a round seal and percentage wound closure, epithelization time and scar area on complete epithelization were measured¹⁷.

Incision wound model: The animals were divided into seven groups of six rats each and kept in separate cages. (Table 2, Fig2) Two Para-vertebral straight incisions of 5 cm length each were made through the entire thickness of the skin, on either side of the vertebral column with the help of a sharp scalpel. After complete homeostasis the wound were closed by means of interrupted sutures placed at equidistance points about 1 cm apart. On the 7th day sutures were removed and on the 10th post-wounding day tensile strength was measured by continuous water flow technique^{18,19}.

Statistical analysis: The mean value ± SEM was calculated for each parameter. Results were statistically analyzed by one-way-analysis of-variance (ANOVA) followed by Dunnett's t-test. p < 0.001 was considered as significant.

RESULTS

The preliminary phytochemical analysis: The preliminary phytochemical analysis of the *C.gigantea* leaves extract showed the presence of cardiac glycosides, alkaloids, steroids, triterpenoids, flavonoids and tannins.

Acute toxicity studies: The methanolic extract of leaves of plant *C.gigantea* was found to be safe up to 1800 mg/kg body weight for methanolic extract and 2000mg/kg body wt. for other extracts by oral route. After 24 h animals were found well tolerated. There was no mortality and no signs of toxicity and extract were found to be safe.

Excision wound model: Topical application of *Calotropis gigantea* increased the percentage of wound contraction and completed wound healing by 18th day, which indicates rapid epithelization and collagenization. In fact, topical administration of methanolic extracts accelerated the progression of wound healing by 12th day, i.e. 70.11 ± 0.54 compared with control 45.09 ± 0.53 . It also reduced the epithelization time from 21.56 to 12.56 days when compared with control and reduced the scar area on complete epithelization from 16.35 to 9.08 mm^2 when compared with control. Povidone iodine showed significant effect, i.e. $p < 0.001$ as compared with control (Table 1).

Incision wound model: The breaking strength of the incision wounds was increased in drug treated groups to significant extent, i.e. 125.48 ± 0.78 in control was increased up to 321 ± 0.99 with methanolic extract. The results are also comparable to standard drug Povidone iodine (Table 2).

DISCUSSION AND CONCLUSION

Wound healing process consists of different phases such as granulation, collagenization, collagen

maturation and scar maturation which are concurrent but independent to each other. Hence in this study two different models were used to assess the wound healing effect of different extracts of leaf of *C.gigantea*. The result of the present study showed that *C. gigantea* possesses a definite prohealing action. In excision wound healing model the methanolic extract of leaf of the plant *C.gigantea* showed significant increase in percentage closure of excision wounds by enhanced epithelization. This enhanced epithelization may be due to the effect of *C. gigantea* extracts on enhanced collagen synthesis. Similarly, the breaking strength of the incision wounds was increased in methanolic extract treated groups in incision wound healing model. Deposition of newly synthesized collagens at the wound site increases the collagen concentration per unit area and hence the tissue tensile strength²⁰. The higher breaking strength indicates better healing of wounds. Higher hydroxyproline content was seen with extract treatment. The increased amount of hydroxyproline in test groups underlines increased collagen content, since hydroxyproline is the direct estimate of collagen synthesis it supports the wound healing activity of *C. gigantea*. Recent studies have shown that phytochemical constituents like flavonoids and triterpenoids are known to promote the wound healing process mainly due to their astringent and antimicrobial properties, which appear to be responsible for wound contraction and increased rate of epithelialization²¹. The preliminary phytochemical analysis of the *C.gigantea* leaf extracts showed the presence of tannins, steroids, flavonoids, cardiac glycosides triterpenoids and alkaloids. The presence of cardiac glycosides and flavonoids as phytochemical constituents in *C.gigantea* may be responsible for the wound healing activity. Hence present research supports traditional claims of the plant in wound healing.

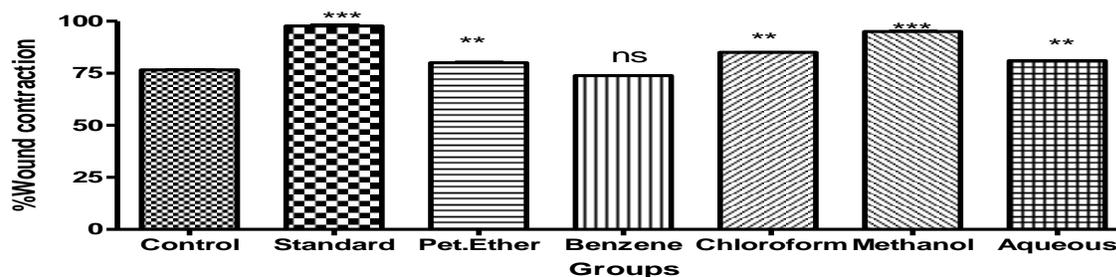


Fig1: Effect of *Calotropis gigantea* leaves extracts topically on excision wound healing model.

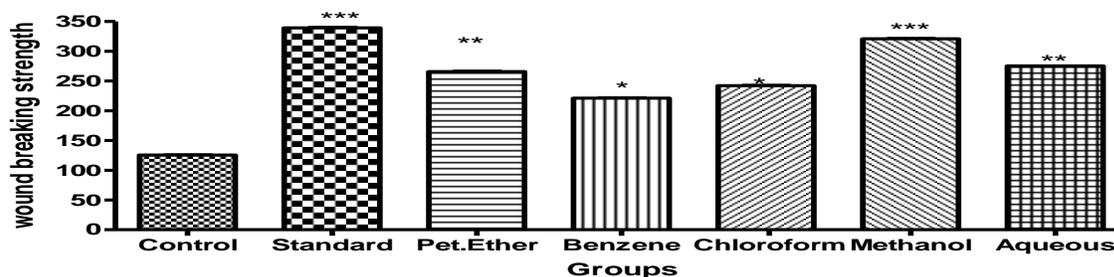


Fig2: Effect of *Calotropis gigantea* leaves extracts orally on incision wound healing model

Table 1: Effect of *Calotropis gigantea* leaves extracts topically on excision wound healing model.

Groups(n=6)	Excision Wound Contraction (mm ²) on days Mean ± SEM						Epithelization (Days)
	% W o u n d C o n t r a c t i o n					Mean Size of Scar area mm ²	
	4th day	8th day	12th day	16h day	18thday		
C o n t r o l	13.90±0.32	24.06±0.29	45.09±0.53	70.57±1.13	76.64±0.24	16.35±0.34	21.56 ± 0.25
S t a n d a r d	45.21±1.05	55.14±0.35	66.42±0.13	80.56±0.64	97.69±0.68*	8.76±0.10*	11.51 ± 0.32
Pet.Ether extract	32.11±0.65	47.21±0.01	61.11±0.43	71.01±0.21	80±0.43*	11.98±0.91*	14.56 ± 0.12 *
Banzene extract	26.66±0.31	43.23±0.12	47.77±0.54	55.01±0.13	73.91±0.03*	13.91±0.55*	16.89 ± 0.32 *
Chloroform extract	39.91±0.45	53.55±1.32	63.11±0.11	78.1±0.76	85.01±0.11*	11.34±0.43*	14.01 ± 0.56 *
Methanol extract	46.64±1.65	54.44±1.63	70.11±0.54	84.12±0.63	95.01±0.32*	9.08±0.63*	12.56 ± 0.56
Aqueous extract	36.76±1.01	48.44±1.43	71.1±0.21	86.54±0.43	81.01±0.21*	13.13±0.43*	13.01 ± 0.21

All values are in Mean ± SEM *P<0.001 Vs control n=Number of animals used

Table 2: Effect of *Calotropis gigantea* leaves extracts orally on incision wound healing model

Groups (n=6)	Wound breaking strength(g)
Control	125.48±0.78
Standard	339.41±1.19***
Pet.Ether extract	265.78±0.98**
Benzene extract	221.3±0.65*
Chloroform extract	242.12±0.89*
Methanol extract	321±0.99***
Aqueous extract	275.41±0.15**

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