

## SCREENING OF WOUND HEALING ACTIVITY OF AERIAL PARTS OF *NARAVELIA ZEYLENICA* (L) DC

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### ABSTRACT

*Naravalia zeylanica* DC (Ranunculaceae) is a climbing vine distributed in hilly areas used by the tribal to cure various ailments such as skin diseases rheumatoid arthritis, wounds and ulcer. From the ethnomedical survey reported that, the powder of the plant was applied externally to treat wounds. Based upon the ethnomedical information the present work focused on screening of wound healing activity for the aerial parts of *Naravalia zeylanica*. The plant material was collected from Kolli hills, Tamilnadu, and authenticated. The plant material was extracted by successive solvent extraction by cold maceration method. The preliminary phytochemical studies confirmed the presence of flavanoids, triterpenoids, alkaloids, polyphenols and saponins in chloroform and ethanolic extract. From the acute oral toxicity studies noted that the chloroform and ethanolic extract did not showed any mortality and toxic reaction up to the dose of 2000mg/kg. The ointments were prepared by using chloroform and ethanol extract in the concentration of 10% w/v. Wound healing activity of *Naravalia zeylanica* was screened by excision wound model in albino rats. From the studies on excision wound model, the results revealed that chloroform extract ointment, ethanolic extract ointment and Povidone Iodine 5% w/w ointment treated animal showed decreased wound area from day to day. on 15th post wounding day, chloroform extract treated animals showed 97.43% of healing and ethanolic extract treated animal showed 96.53% where as standard treated animal showed 99.13% healing (Figure no. 44-49). All readings were found to be statistically significant and comparable (\*p<0.05, \*\*p<0.01). The wound healing property of *Naravalia zeylanica* appears to be due to the presence of its active principles, which accelerates the healing process. Polyphenol, alkaloids and saponins compound may be responsible for wound healing activity.

**Keywords:** *Naravalia zeylanica*, wound healing activity, excision wound model.

### INTRODUCTION

Wound healing, or wound repair, is an intricate process in which the skin repairs itself after injury. The plant selected for the present study on the basis of endangered species, ethno medical information towards wound healing property. *Naravalia zeylanica* DC (Ranunculaceae) is a woody climber with tuberous roots opposite, ovate, cordate leaflets, small flowers arranged in panicles and

red coloured achenes along with long feathery styles, occurring in the hot to warm regions in India<sup>1</sup>. The whole plant is traditionally used in vitiated vata, pitta, inflammation, skin diseases, wounds and ulcers. Leaf paste is consumed to treat Chest pain. Stem paste of *Naravalia zeylanica* applied for itches, scabies and allergies. The powder of the plant was applied externally to treat wounds and ulcers. Based upon the ethnomedical importance,

present work focused to reveal the wound healing property of aerial parts of *Naravalia zeylanica*<sup>1-5</sup>.

## MATERIALS AND METHODS

### Collection and Extraction of Plant Material

*Naravalia zeylanica* DC was collected from Kolli hills of Namakkal district, Tamilnadu, India. The plant was identified and authenticated by National Institute of Herbal Science, Chennai<sup>7-8</sup>. The aerial part of the plants were collected in the month of August and shade dried. The coarse powder of the aerial parts of the plant material was extracted by cold maceration method using successive solvents such as petroleum ether, chloroform and ethanol in increasing polarity for 48 hours each. The extracts were concentrated by distilling the solvent and dried under reduced pressure.

### Preliminary Phytochemical Tests

Petroleum ether, chloroform, ethanol and aqueous extracts were subjected to qualitative phytochemical chemical tests to identify the phytoconstituents using standard reagents<sup>9-10</sup>.

### Acute Toxicity Study

The animal studies were carried out after getting approval from the institutional animal ethical committee. Acute oral toxicity was performed as per Organization for Economic Co-operation for Development (OECD) guideline 423 methods. Healthy young adult Swiss albino mice, weighing about 25-30gm of either sex were divided into three groups (chloroform extract, ethanolic extract & control) of each nine animals. The chloroform and ethanolic extracts was administered in a single dose by gavage using specially designed mice oral needle. Animals were fasted 3 h prior to dosing. Following the period of fasting animals was administered orally at a single dose of 2000 mg/kg. After substance administration, food was withheld 2 hrs in mice. Animals were observed individually after at least once during the first 30 minutes, periodically during the first 24 hrs, with special attention given during the first 4 hrs, and daily thereafter, for a total of 14 days. The direct observation parameters such as Tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma were observed.

### Screening of Wound Healing Activity

#### Preparation Extract Ointment

For the screening of wound healing activity, the ointments were prepared by using chloroform and ethanol extract. The chloroform and ethanolic extract ointment

were prepared in the concentration of 10% w/v, by using Polyethylene Glycol (PEG) Ointment base (Mixture of PEG 4000 and PEG 600 in the ratio of 3:7).

### Excision Wound Model<sup>11-12</sup>

The experimental protocols were approved by the Institutional Animal Ethical Committee (IAEC). Male albino Wistar rats weighing 150 gm were divided in to four groups of 6 rats each. Group I animals were considered as the control. Grouped II animals were served as the standard. Group III & IV animals were treated chloroform and ethanolic extract test animals. Animals were anaesthetized with anesthetic ether and placed in operation table in its natural position. A square wound of about 1.5 cm (width) x 0.2 cm (depth) was made on depilated ethanol-sterilized dorsal thoracic region of rats. Infection was made on wound by inoculating *Staphylococcus aureus* and separated the animals into groups. Group I animals were considered as the control. Grouped II animals were served as the standard and were treated with Povidone Iodine 5% ointment, Group III & IV animals were treated with ointment prepared from chloroform and ethanolic extract topically. Povidone Iodine 5% ointment and extract ointments were topically applied once a day, till the epithelialization was complete. The wound contraction was studied by tracing the raw wound area subsequently on day 3, 5, 7, 9, 12, 15 on graph paper. Scar area and time of complete epithelialization were also measured. The percentage of inhibition (wound closure) and period of epithelialization were recorded.

## RESULTS

The aerial part of *Naravalia zeylanica* was extracted with solvents such as petroleum ether, chloroform and ethanol successively by cold maceration method. From the preliminary phytochemical test, presence of triterpenoids, alkaloids, flavonoids and saponins were observed in chloroform and ethanolic extracts. There was no death other complications reported in the 14 days of acute oral toxicity studies up to dose 2000mg/kg. The effective dose (ED<sub>50</sub>) was assigned as 200mg/kg. Wound healing activity of *Naravalia zeylanica* was screened by excision wound model in albino rats. The wound contraction was studied by tracing the raw wound area subsequently on day 3, 5, 7, 9, 12, 15 on graph paper. Scar area and time of complete epithelialization were also measured. The percentage of inhibition (wound closure) and period of epithelialization were recorded

(Table no. 1). From the studies on excision wound model, the results revealed that chloroform extract ointment, ethanolic extract ointment and Povidone Iodine 5% w/w ointment treated animal showed decreased wound area from day to day (Figure no. 50-53). However, on 15th post wounding day, chloroform extract treated animals showed 97.43% of healing and ethanolic extract treated animal showed 96.53% where as standard treated animal showed 99.13% healing (Figure no. 44-49). All readings are found to be statistically significant and comparable (\* $p < 0.05$ , \*\* $p < 0.01$ ).

## DISCUSSION

Wound healing involves various phases. Initially involves acute inflammatory phase followed by the synthesis of collagen and other extra cellular macromolecules, which are later removed to form a scar<sup>149</sup>. The epithelization time i.e. time at which complete scar formation occurs, also suggest that chloroform extract

treated group found to be significant and comparable with control. The increased rate of wound contraction and decrease in period of epithelization in the animals treated with chloroform extract may be attributed to their broad spectrum antibacterial activity. The enhanced capacity of wound healing may be on the basis of anti-inflammatory effects of the plant. The wound healing property of *Naravelia zeylanica* appears to be due to the presence of its active principles, which accelerates the healing process. Polyphenol, alkaloids and saponins compound may be responsible for wound healing activity.

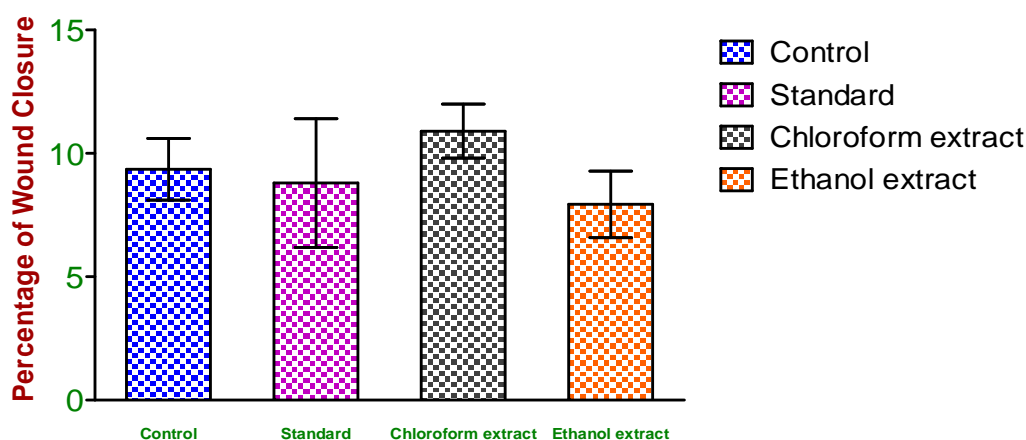
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**Table 1: Effect of *Naravelia zeylanica* extracts on excision wound model**

Day	PERCENTAGE OF WOUND CLOSURE			
	Control	Standard	Chloroform extract	Ethanol extract
3	9.35 ± 1.24	8.79 ± 2.60	10.90 ± 1.09	7.93 ± 1.34
5	16.45 ± 1.50	17.76 ± 3.87	19.43 ± 0.95	16.01 ± 2.08
7	25.56 ± 3.02	49.18 ± 3.07**	39.13 ± 5.26*	38.43 ± 2.61*
9	41.95 ± 5.55	65.38 ± 3.65**	55.51 ± 3.67*	48.50 ± 2.21
12	70.65 ± 5.17	91.83 ± 0.96**	87.43 ± 1.61**	84.93 ± 1.41**
15	86.36 ± 1.32	99.13 ± 0.40**	97.43 ± 0.57*	96.53 ± 0.18
Epithelization (Days)	24	17	18	20

Values are expressed as mean ± SEM. \*\* $p < 0.01$ , \* $p < 0.05$ .  
(One-way ANOVA followed by Dunnett's test)



**Fig. 1: Effect of *Naravelia zeylanica* extracts against excision wound model on the 3<sup>rd</sup> day of treatment**

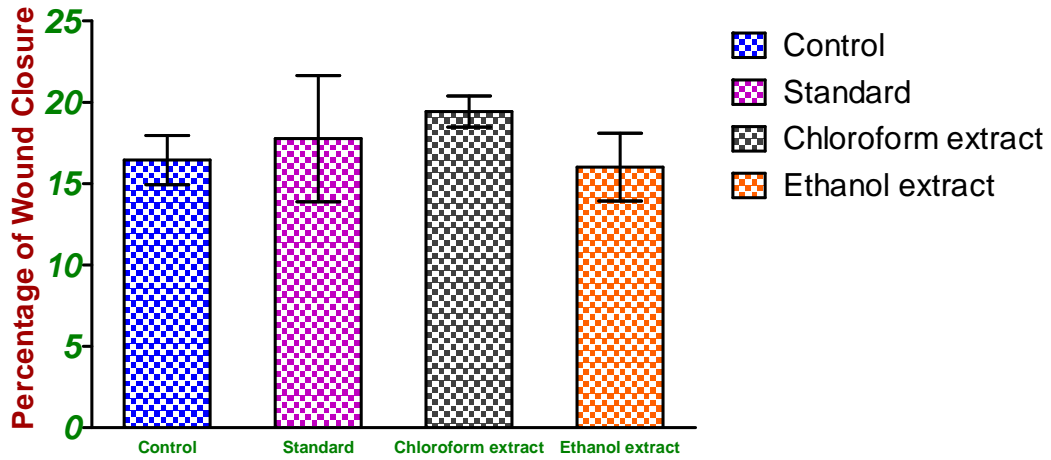


Fig. 2: Effect of *Naravelia zeylanica* extracts against excision wound model on the 5<sup>th</sup> day of treatment

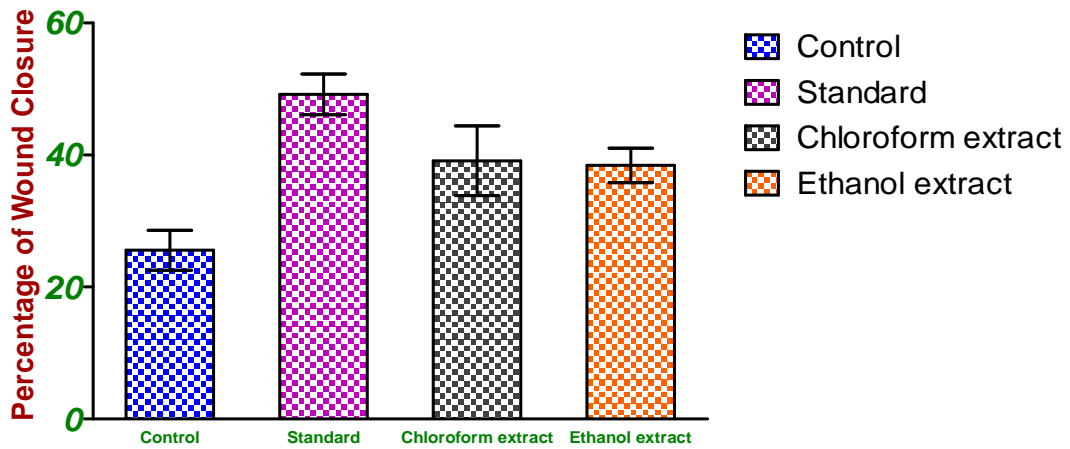


Fig. 3: Effect of *Naravelia zeylanica* extracts against excision wound model on the 7<sup>th</sup> day of treatment

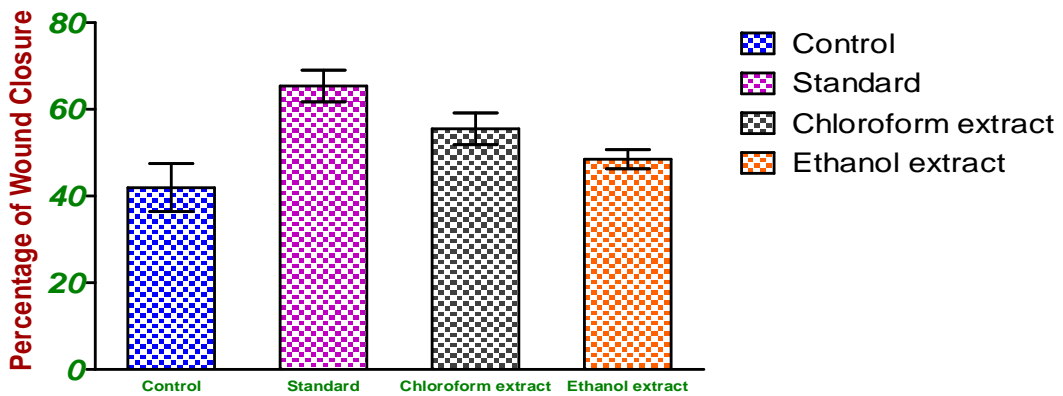


Fig. 4: Effect of *Naravelia zeylanica* extracts against excision wound model on the 9<sup>th</sup> day of treatment

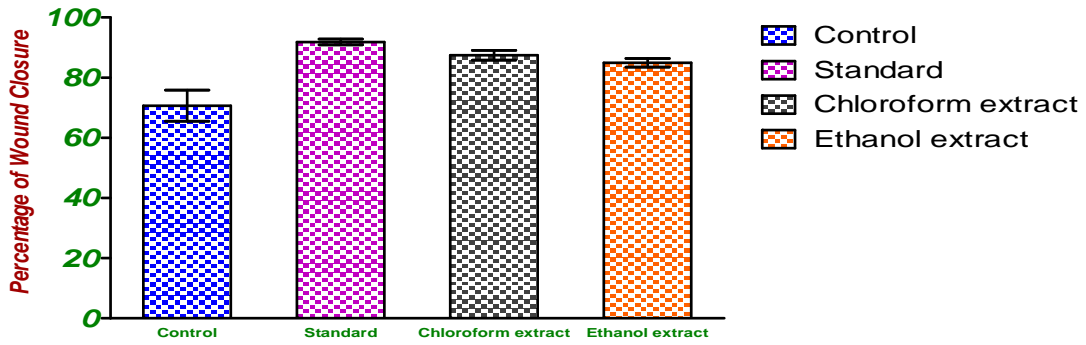


Fig. 5: Effect of *Naravelia zeylanica* extracts against excision wound model on the 12<sup>th</sup> day of treatment

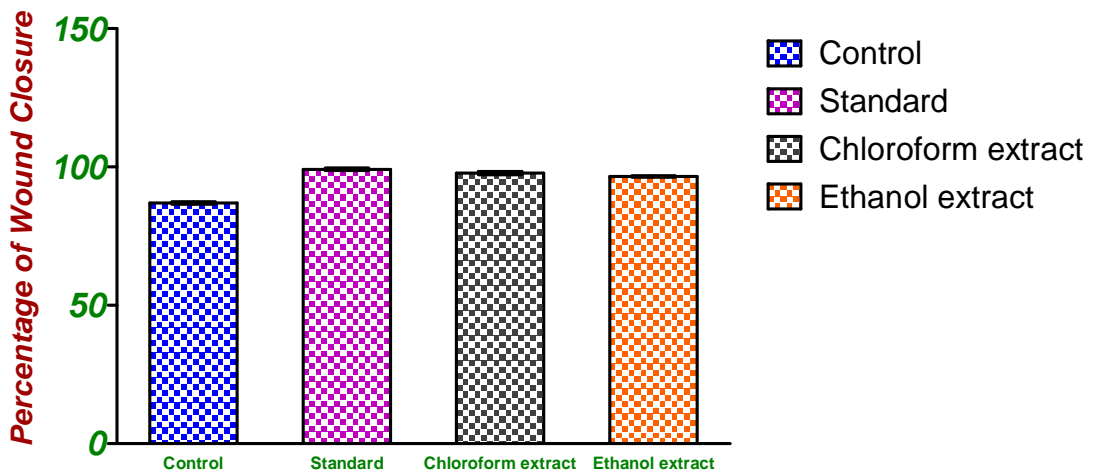


Fig. 6: Effect of *Naravelia zeylanica* extracts against excision wound model on the 15<sup>th</sup> day of treatment

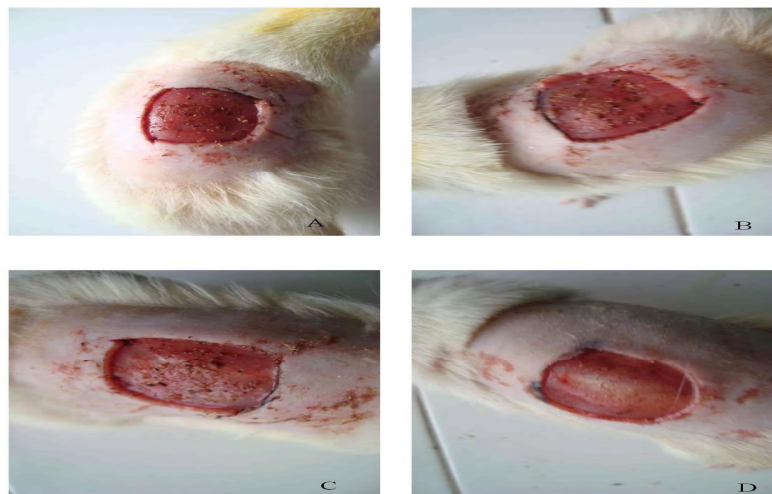
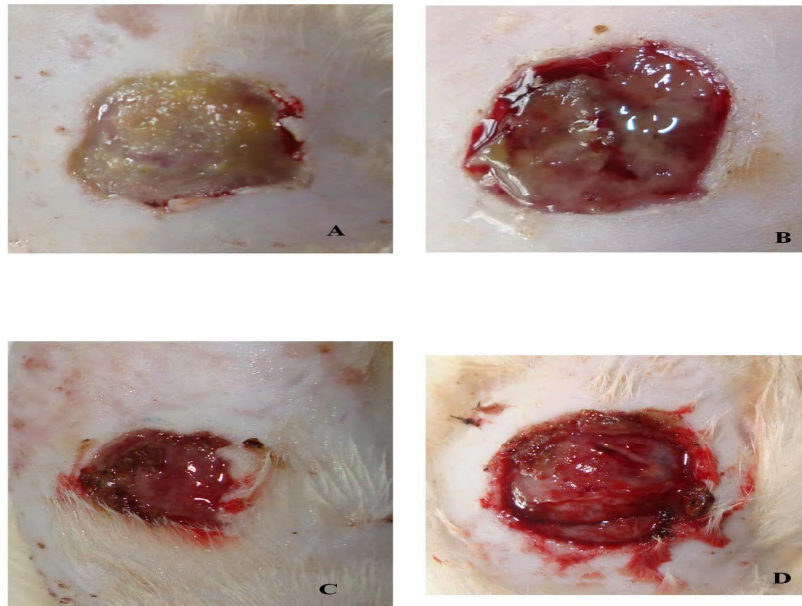
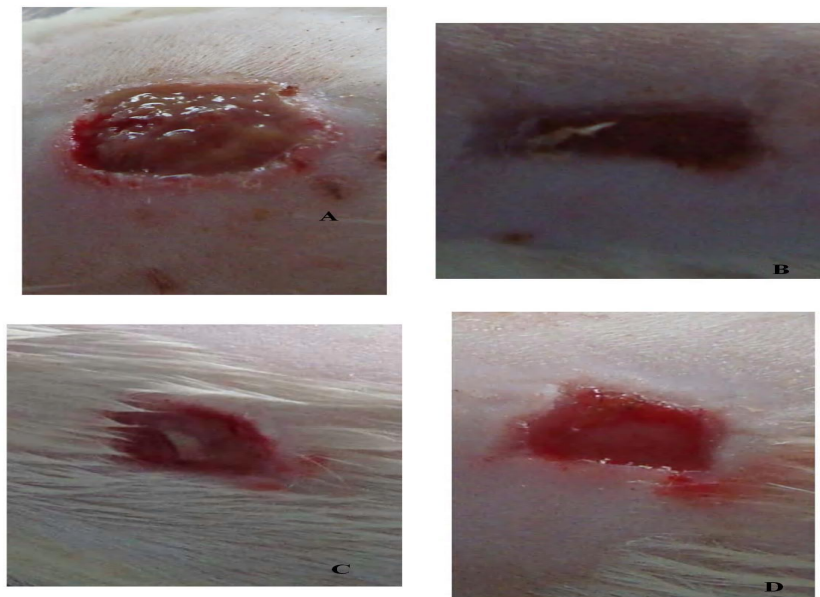


Fig. 7: Macroscopic observation of excision wounds on day-1

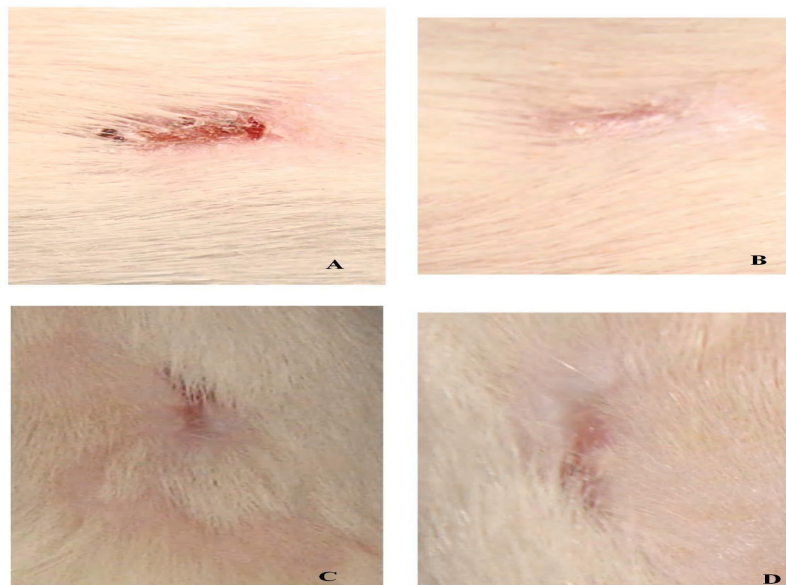


**Fig. 8: Macroscopic observation of excision wounds on day- 4**



**Macroscopic observation of excision wounds on day- 16**





**Macroscopic observation of excision wounds on day- 21**  
**A-Group I – Normal control, B- GroupII- Standard, C-GroupIII- Chloroform extract, D-Group IV-Ethanolic extract**

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