Research Article

Wound healing activity of *Euphorbia antiquorum* stem extract on rats

B. Sudheer Chowdary*, M. Sathish Kumar, M. Vamsi Krishna, K. Vikram Kumar, M. Jesu Mercy, V. Mohan Aditya, P. Tejasri

Department of Pharmacology, Bapatla College of Pharmacy, Bapatla, Guntur (Dt), Andhra Pradesh, India

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Abstract

Background: Wounds are inescapable events of life which are due to physical, chemical and microbial infections. Medicinal plants came into prominence having good wound healing activity with low adverse drug reactions than the synthetic drugs. **Objective:** The present study was designed to evaluate the wound healing activity of *Euphorbia antiquorum* ethanol stem extract formulation on the wistar rats by using excision wound model. **Materials and Methods:** Animals were divided into four groups containing six animals each. Excision wound was made on the back of rats near the neck region about 2mm diameter. The *Euphorbia antiquorum* ethanol extracts were formulated into ointment by using hydrophilic base containing 2.5 and 5 percentage strength. The formulations were applied on the wounds twice a day and wound healing was observed on the 5, 10, 15, 20th days. Wound contraction period, percentage of inhibition and bio chemical parameters were observed. **Results:** The wound contraction for the *Euphorbia antiquorum* 2.5% ointment was 45.76% ±3.07 to 98.05% ±0.24 and 5 % ointment was 72.45% ±3.43 to 99.13% ± 0.25. Significant wound contraction was observed in 5% strength containing formulation when compared with control and standard. **Conclusion:** Thus from the present study *Euphorbia antiquorum* ethanol formulation (5% ointment) showed significant wound healing activity compared with standard and control groups.

***Keywords*:** Wound healing, *Euphorbia antiquorum*, excision wound model, ethanol extract

Introduction

Wound is defined as disruption of the cellular and anatomical continuity at a tissue. It may be produced by physical, chemical, thermal and microbial damage to tissue. A large number of herbs are used as a folklore medicine for the treatment of cuts, wounds, and burns. In India there is a surplus number of variety of species of plants present which can be used in the treatment of various wounds (Biswas et al., 2003).

The wound healing process consists of three phases. First phase includes homeostasis which often occurs immediately after the wound occurs with vascular constriction and aggregation of platelets. Second phase is inflammatory phase characterized by the five common signs i.e redness, pain, heat, swelling and loss of function. All the inflammatory mediators are produced in this phase cause chemotaxis of monocytes, lymphocytes and initiates epithilisation process. The last phase is remodeling phase characterized by increasing the strength of scar and cause complete reepithilisation (Mutsaers et al., 1997). Various factors like oxygen, age, sex hormones, stress, will affect the wound healing process (Sabale et al., 2012).

The *Euphorbia antiquorum* stem consists of various phytochemical constituents that may responsible for wound healing. This plant is topical plant widely distributed over the world ranging from annual weeds to trees. The plant contains latex, green stem, ribs are prominent generally three. The characteristic feature is triangular in shape. Flowers are cyathia yellowish green to pinkish in colour (Wine et al., 2011). Male flowers with one stamen, female flower lies at the center of cyathium. Fruits are yellowish to orange in colour. It contains various chemical constituents like flavonoids, di & tri terpenes which shows a significant activity of antimicrobial, anti HIV and cytotoxic properties (Sivaraj et al., 2011; Sumathi et al., 2012). The latex shows the lavicidal and mosquitocidal activity(Vimal et al., 2014; Vimal et al., 2015).

Address for Corresponding Author:
B. Sudheer Chowdary
Bapatla College of Pharmacy, Bapatla - 522101, Guntur (Dt), Andhra Pradesh, India
E- Mail: sudheer.chowdary18@gmail.com

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Materials and Methods

Plant material and collection

Stem of *Euphorbia antiquorum* was collected around Machilipatnam, Krishna District and authenticated by Botanist.

Extraction

Euphorbia antiquorum stems were collected washed thoroughly, then cut into small pieces. The pieces were dried under the shade for 3-4 days. The dried stem material was pulverized into a coarse powder. The powder was weighed and debris is removed. About 150gm of powder was weighed and subjected to maceration process with various solvents depends up on the polarity. The mixture is occasionally stirred vigorously and kept for 72 hrs. Finally the marc was filtered through a muslin cloth and the filtrate was concentrated by using rotary evaporator. The obtained filtrate was subjected to phytochemical screening for the identification of chemical constituents (Kumar et al., 2016).

Preparation of ointment

The water soluble base was prepared by triturating the PEG – 400 & PEG – 4000 in a motor. 2.5% and 5% ethanolic extract containing ointment was prepared (Esimone et al., 2006; Jyothi et al., 2014).

Screening of wound healing activity

The study was approved by the Institutional Animal Ethics Committee with Ref no: IAEC/XI/3/BCOP/2017. Adult wistar rats weighing 200-250 gm of either sex was selected for the study and maintained under standard experimental conditions as per CPCSEA guidelines. Animals were divided into 4 groups containing six animals each. Group – 1 was treated as control, Group -2 was treated as standard (Povidine Iodine 5%), Group – 3 was treated with 2.5% *Euphorbia antiquorum* ethanolic extract ointment, Group – 4 treated with 5% *Euphorbia antiquorum* ethanolic extract ointment. Excision wound was made on the back of rats near the neck region. The region was fully shaved and made a incision about 2mm diameter. The wound was left undressed to the open environment (James et al., 2010). To the standard and test group animals drugs was topically applied for 24 days. The wound area was observed at 5, 10, 15, 20th day and the parameters like wound contraction period and percentage of inhibition were monitored (Mathew et al., 2004; Umachigi et al., 2008).

Biochemical estimation

To estimate collagen and hexosamine, the tissue samples were defatted in chloroform methanol (2:1) and dried in acetone before use. Collagen, hexosamine and uronic acid was estimated by the method of Anusha (Bhaskar et al., 2012).

Results and discussion

The phytochemical screening of various solvent extracts of *Euphorbia antiquorum* was carried out and the results are given in the table 1. The ethanol extract was selected for further pharmacological screening due to quantity and the presence of flavonoids, tannins and saponins.

The formulation (2.5% and 5%) was subjected to different tests like pH, skin irritation test, colour, odour and their results is shown in table 2.

For the determination of wound healing activity the wound size for all animals groups was measured in mm^2. The percentage wound contraction of the control changed from 5th to 20th day is 21.22% ± 6.54 to 92.86% ± 1.30. For the standard it is from 54.85% ± 3.08 to 99.6% ± 0.10. The *Euphorbia antiquorum* formulation (2.5%) showed a change from 45.76% ± 3.07 to 98.05% ± 0.24 from 5th to 20thday. *Euphorbia antiquorum* formulation (5%) showed an increased percentage wound contraction from 72.45% ± 3.43 to 99.13% ± 0.25 from 5 to 20thday.

The effect of *Euphorbia antiquorum* on the reduction of the wound area (in mm^2) for 0 to 20th day is given in the figure 1 & 2. The 5% ointment showed significant decrease in the wound size when compared with control group. Biochemical changes were evaluated on 19th day and the results are tabulated as (Table 3).

Table 1. Phytochemical analysis of *Euphorbia antiquorum* extract

S. No	Solvent s	TE	ST	FL	TA	PH	SA	CG	CA	AP	AL
1	Petroleum Ether	-	-	+	-	-	+	-	-	-	-
2	Chloroform	-	-	+	+	+	-	-	+	-	+
3	Benzene	-	-	+	-	-	-	-	+	-	-
4	Ethanol	-	-	+	+	-	+	-	+	-	-
5	Water	-	-	+	-	-	+	-	-	-	+

TE: Terpenoids; ST: Steroids; FL: Flavanoids; TA: Tannins; PH: =Phenols; SA: Saponins; CG: Cardio Glycosides; CA: Carbohydrates; AP: Aminoacids; AL: Alkaloids

Table 2. Properties of the ointment

S. No	Test Performed	Ointment (2.5%)	Ointment (5%)
1	pH	6	6
2	Irritability Test	No skin irritability was found	No skin irritancy was found
3	Colour	Light green	Green to dark brown
4	Odour	Characteristic	Characteristic
5	Homogenesity	No clumps were found	No clumps were found

Table 3. Biochemical parameters

S. No	Total collagen	Hexosamine	Uronic acid
Control	2.983±0.29*	353.3±2.15	84.16±1.16
Standard	3.96±0.19	310.0±10.65**	80.83±0.60
Test 2.5	3.63±0.25	315.8±6.11**	80.0±1.52
Test 5	3.58±0.23	315.8±9.62**	77.83±1.93*

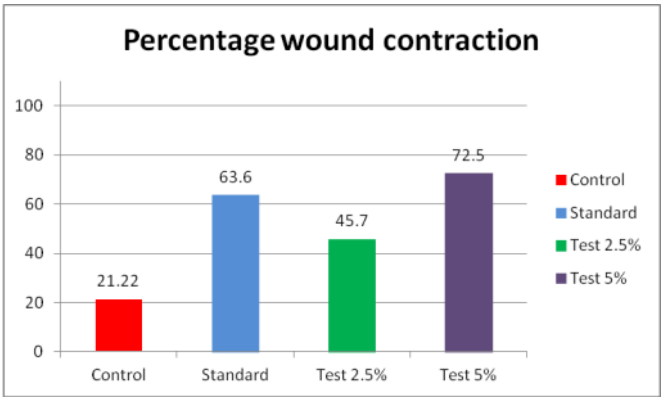

Figure 1. Percentage Wound Contraction on Day 5

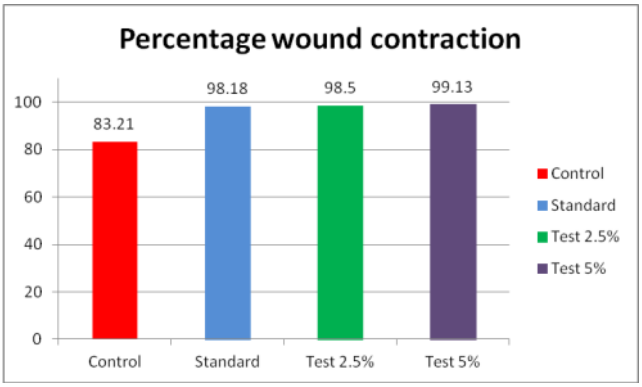

Figure 2. Percentage Wound Contraction on Day 20

Conclusion

The present study evaluated the wound healing activity of *Euphorbia antiquorum* ethanol extract using excision wound model in rats. The regenerated skin in the 5% ointment treated rats showed the best healing features compared to the control and 2.5% ointment treatment groups. Therefore it is concluded that ethanol stem extracts of *Euphorbia antiquorum* possesses wound healing activity.

Conflicts of interest: Nil

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