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Investigation on the wound healing activity of aqueous extract of *Emilia sonchifolia* (L.) Dc

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Abstract

Search for a natural herb with perfect Wound healing effect was in progress for many years. *Emilia sonchifolia*, Family: Asteracea, commonly known as cupid's shaving brush or lilac tassel flower, is a soft annual herb used for healing wounds as a folk medicine. From literature review it was found that most of the tribal people are using this for wound healing. So the present study aims to evaluate the pharmacological activity of the aqueous extract of *Emilia sonchifolia* using Male albino Wistar rats Excision and incision wound model. The plant has showed good wound healing activity.

Keywords: *Emilia sonchifolia*, wound healing activity, traditional use, incision method.

1. Introduction

A large number of medicinal plants were found to have wound healing activity in traditional system of medicine. Although these herbs are used since ancient time, its efficacy is not proven scientifically. *Emilia sonchifolia* is a soft annual herb which grows up to 40 cm in height, leaves are simple, lyrate-pinnate with large terminal lobe, flowers purplish with corymbose heads. Fruits are oblong containing many seeds. The plant occurs in open field and waste lands. The plant is effective in treatment of fever, tonsillitis. The juice is used for eye infections. The herb is useful in conditions like cough, bronchial disorder, piles, worm infections, diarrhea, swelling and diabetics. The other reported activities of the plant includes anti anxiety, hepato protective, anti cataract and anti convulsant activities. Tribal uses the juice of crushed whole plant for wound healing ^[1].

The phyto chemical screening of drug shows that the plant is rich with flavonoids and flavone glycosides. The aerial part contains two pyrrolizidine alkaloids identified as senkirkine and doronine. It also contains vitamins like riboflavin and niacin. This plant is considered as one among the ten sacred flowers of Kerala state commonly known as 'Dasapushpam' which are predominantly used in Ayurveda ^[2].

Wounds are injuries that break the skin or other body tissues. They include punctured skin, cuts, scratches, and scrapes. A large number of plants/plant extracts are used by tribals and folklore traditions in India for treatment of wounds. Plants /its active constituent derived from plants are need to be identified and formulated for the treatment of wounds. In this view a number of plant drugs are being investigated at present. Plants have immense potential in the treatment of wounds ^[3].

Wound healing is a natural restorative response to tissue injury. Healing is the process of a complex cascade of cellular events that leads Healing is a systematic process, generally explained in terms of 4 classic stages that is 1) hemostasis, 2) inflammation, 3) proliferation, and 4) maturation ^[4].

Hemostasis

It is the first step or process of the wound being closed by clotting. It starts with when blood leaks out of the body tissue. In this step of hemostasis blood vessels constrict to restrict the blood flow. Next step, blood platelets stick together to seal the break in the wall of the blood vessel. Finally, coagulation occurs and reinforces, platelets are plug with threads of fibrin. The hemostasis stage happens very quickly.

Inflammation

It is the second stage of wound healing and begins after the injury when the injured blood vessels leak transudate causing localized swelling. Inflammation controls both bleeding and infection. During this inflammatory phase, damaged cells, pathogens, and also bacteria are

removed from wound surface. This stage of wound healing is characterized by pain, redness. Inflammation is the natural part of the wound.

Proliferative Phase

This phase of wound healing is when wound is rebuilt with new tissue made up of collagen and extracellular matrix. In proliferative phase, the wound tissue contracts as new tissues are built. And a new network of blood vessels constructed so the granulation tissue can be healthy and receive sufficient oxygen and nutrients.

Maturation Phase

This phase is also known as remodeling stage of wound healing, the maturation phase is when collagen is remodeled from type III to type I and the wound fully closes. The cells that had been used to repair the wound. During the maturation phase, collagen is aligned along with tension lines, at the same time water is reabsorbed so that collagen fibers can lie closer and cross-link. Cross-linking of collagen fibers reduces scar thickness and makes the skin of wound stronger [5].

There are two types of Factors influencing wound healing. Local Infection by tissue organization, Poor blood supply to the affected part, Movement of affected part, Exposure to ionizing radiation and inclusion of foreign bodies. Systematic factors like, healing is rapid in young age and slow in old people, Nutritional deficiency of vitamin C and zinc, Haematological abnormalities affects healing and Diabetics [4].

2. Materials and methods

2.1 Plant material

The plant *Emilia sonchifolia* (L.) DC was collected from local area of Malappuram District, Kerala, India during July-August 2016.

2.2 Preparation of extract

The whole plant was collected, crushed and aqueous extract were prepared and concentrated under reduced pressure.

2.3 Preliminary phytochemical analysis

The aqueous extract was tested qualitatively for various active constituents according to standard methods [6].

2.4 Animals

Male albino Wistar rats weighing 110 to 150g were used in this study. All the studies conducted were approved by the institutional Animal ethical committee of Al Shifa College of pharmacy, Kerala, India. (1195/ac/08/CPCSEA) According to prescribed guidelines of committee for the purpose of control and supervision of experiments on animals.

2.5 Wound healing activity

2.5.1 Excision wound model

Excision of wounds was made as described by Morton and Malone (1972) [7]. Animals were anaesthetized with anesthetic ether and placed in dissection 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. Made infection on wound by staphylococci aureus and separate the animals in to groups. Male albino Wistar rats weighing 110 to 150g 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 and were treated with cipladin ointment; Group III animals were treated with 500mg/kg body weight of aqueous extract. Cipladin and

plant extract 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 1, 3, 5, 7, 9, 12, 15 on graph paper. Scar residue, area and time of complete epitheliasation were also measured. The percentage of wound closure and period of epitheliasation were recorded [7].

2.5.2 Incision Method

Group I-control

Group II-Standard drug treatment (Cispladin)

Group III-Sample 10%AEES treatment (500mg/Kg body weight)

Group IV-Sample 20%AEES treatment (500mg/Kg body weight)

The incision wound model was studied after anaesthetizing the animal with ether. The animals were kept in the operation table in its natural position. One paravertebral straight incision of six centimeter was made on either side of vertebral column with the help of scalpel blade. 70% alcohol soaked cotton balls are used for cleaning the wounds. Kept the animals in separate cages. The extract were applied at the dose of 500mg/Kg as gels body weight 10 days. Sutures were removed on the ninth day of post wounding. Tensile strength was measured on the 10th day by continuous constant water supply technique [8,9].

2.6 Data analysis

All the data were subjected to statically analysis using ANOVA.

3. Result

Table 1: Percentage yield of AEES

Solvent	Color and consistency	Percentage yield
50% Water and 50% alcohol	Dark green and semisolid	15.4%

Table 2: Preliminary phytochemical screening of AEES

Phytochemical constituents	Aqueous extract
Carbohydrate	+
Glycosides	+
Phytosterols	-
Flavonoids	+
Alkaloids	-
Tannins	-
Triterpenoids	-
Saponins	+
Proteins	-

Both the concentration of the test samples AEES (10% and 20%) does not showed any irritation inflammation and redness.

Table 3: Skin irritation study of AEES

Group	Sign	Score
Control	-	0
10%AEES	No marked redness and inflammation	0
20%AEES	No marked redness and inflammation	0

Wound Healing Activity

A marked decrease in the period of epitheliation was observed after 10% and 20% AEES application compared to control group. About 50% decrease was observed. Cipladine shows

significantly reduced period of epitheliation compared with control group.

Table 4: Effect of AEES Extract of *Emilia sonchifolia* on excision wound (wound area mm²)

Day	Control	Standard	AEES 10mg/kg	AEES20mg/Kg
3	9.358334 ± 1.247959	8.799999 ± 2.60717	10.900 ± 1.096358	12.194 ± 0.987
5	16.450 ± 1.509249	17.76667 ± 3.87588	21.150 ± 2.425249	22.427 ± 1.764
7	25.56667 ± 3.025962	49.18333 ± 3.07684**	34.38333 ± 2.133294*	34.425 ± 1.967*
9	41.950 ± 5.552822	65.38333 ± 3.657997**	49.66667 ± 1.844572	50.535 ± 1.554
12	70.650 ± 5.173506	91.83334 ± 0.967356**	84.61667 ± 2.474458**	85.250 ± 2.065**
15	94.36667 ± 1.329829	99.13333 ± 0.4046942**	96.550 ± 0.3621702	96.670 ± 0.921

All the values are expressed as mean ± SEM. *p<0.05, **p<0.01. (One way ANOVA followed by Dunnett's Test)

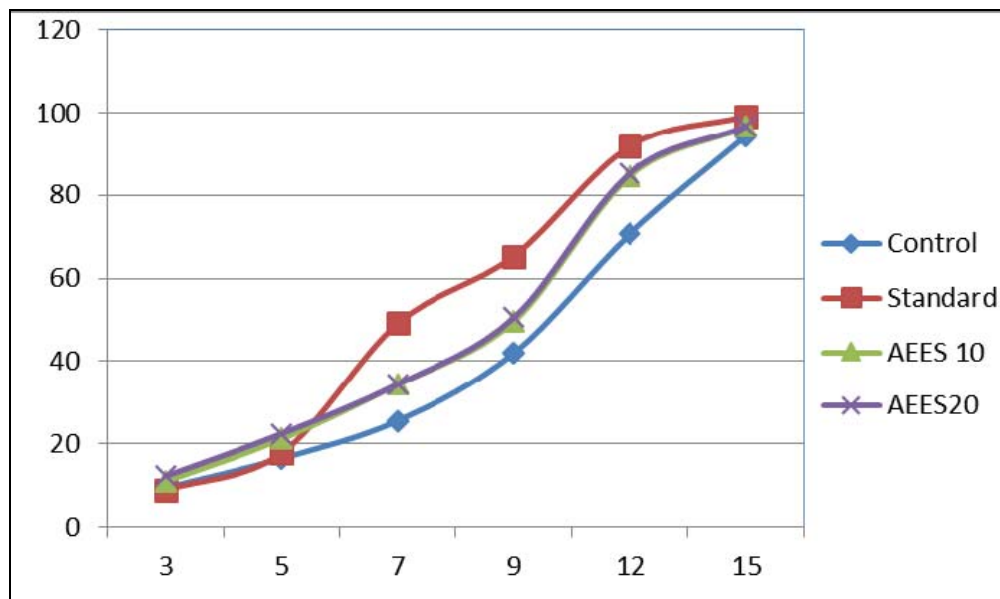


Fig 1: Effect of AEES Extract of *Emilia sonchifolia* on excision wound (wound area%)

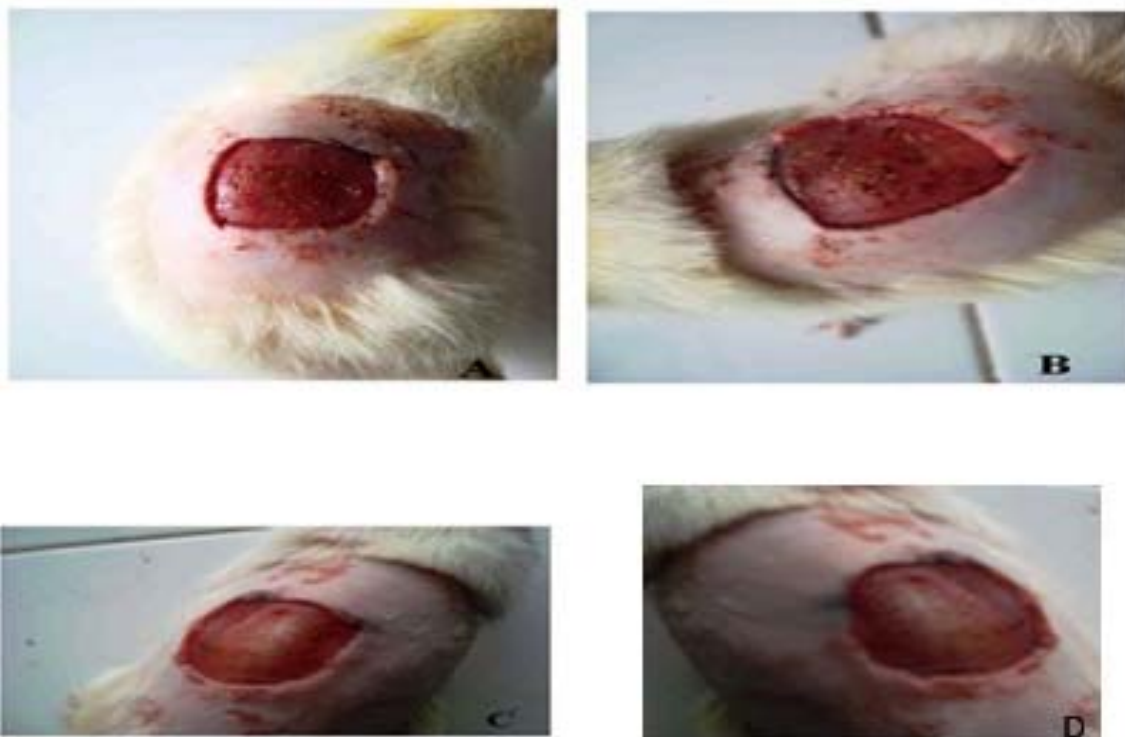


Fig 2: Macroscopic observation of excision wounds on day- 1. A- Group I – Normal control, B- Group II- Cipladine, C-Group III-10% AEES extract, D-Group IV- 20% AEES extract.

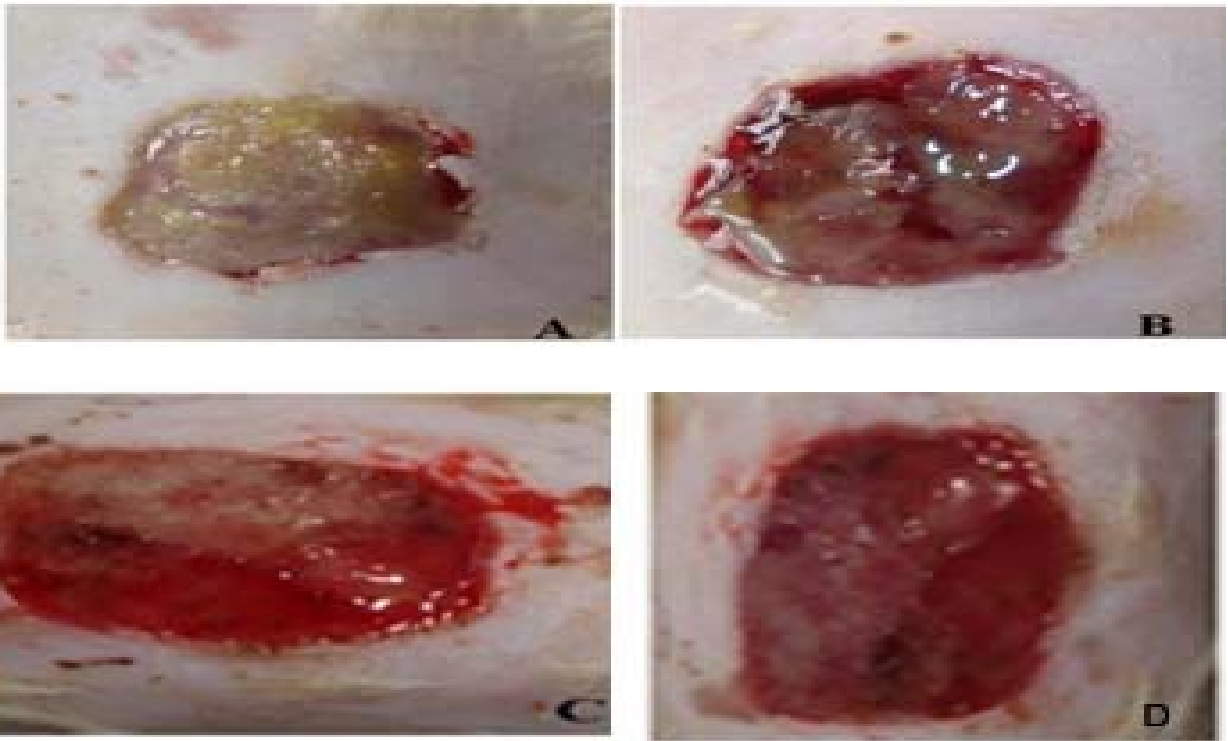


Fig 3: Macroscopic observation of excision wounds on day- 4. A-Group I – Normal control, B- GroupII- Cipladin, C-Group III- 10% AEES extract, D-Group IV- 20% AEES extract.

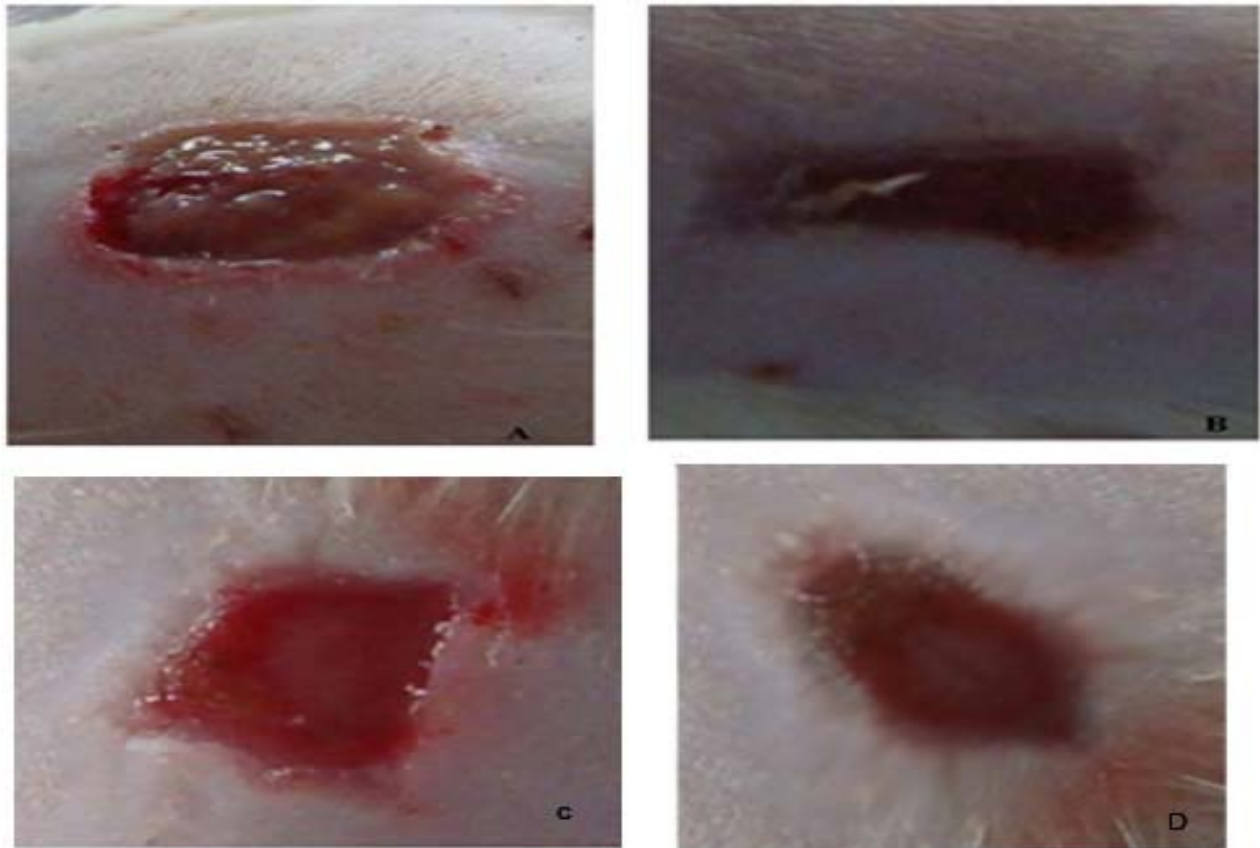


Fig 4: Macroscopic observation of excision wounds on day- 16. A-Group I – Normal control, B- GroupII- Cipladin, C-Group III- 10% AEES extract, D-Group IV- 20% AEES extract.

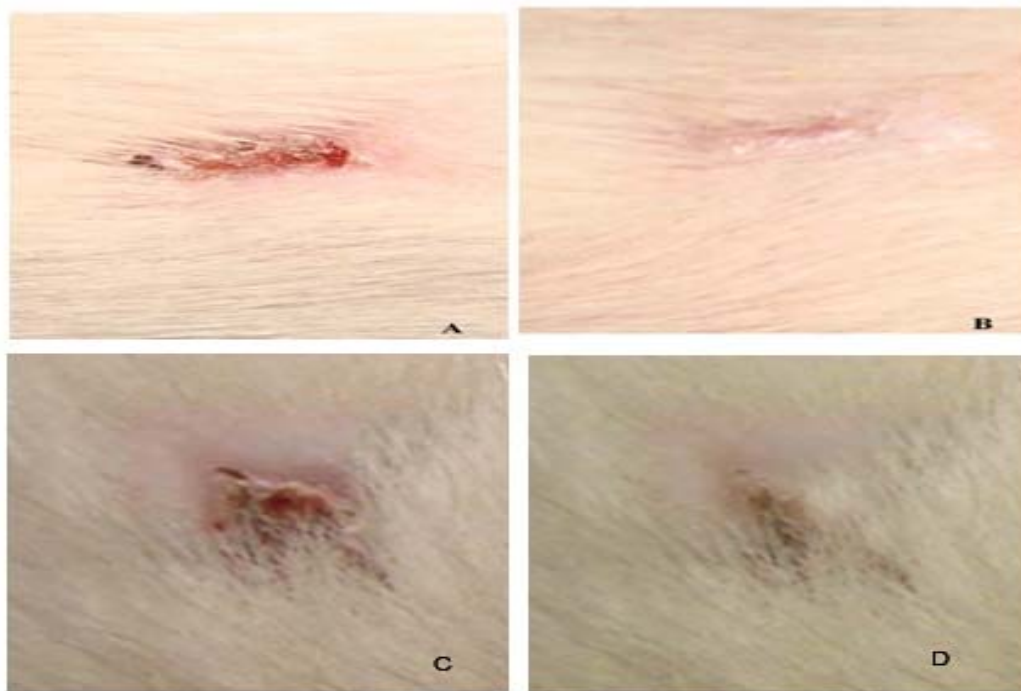


Fig 5: Macroscopic observation of excision wounds on day- 21. A-Group I – Normal control, B- GroupII- Cipladin, C-Group III-

Fig 5: Effect of hydroalcoholic extract of *Emilia sonchifolia* on wound breaking strength (g) in incision wounds

Groups	Treatment	Wound breaking strength
GROUP I	-----	267.31 ± 2.902*
GROUP II	CIPLADINE	355.16 ± 1.904*
GROUP III	10 % EXTRACT	328.01 ± 1.48*
GROUP IV	20 % EXTRACT	357.45 ± 2.29*

Values are mean ± SEM (n-6) oneway ANOVA followed by Dunnet’s test where * represents significant at < 0.05.

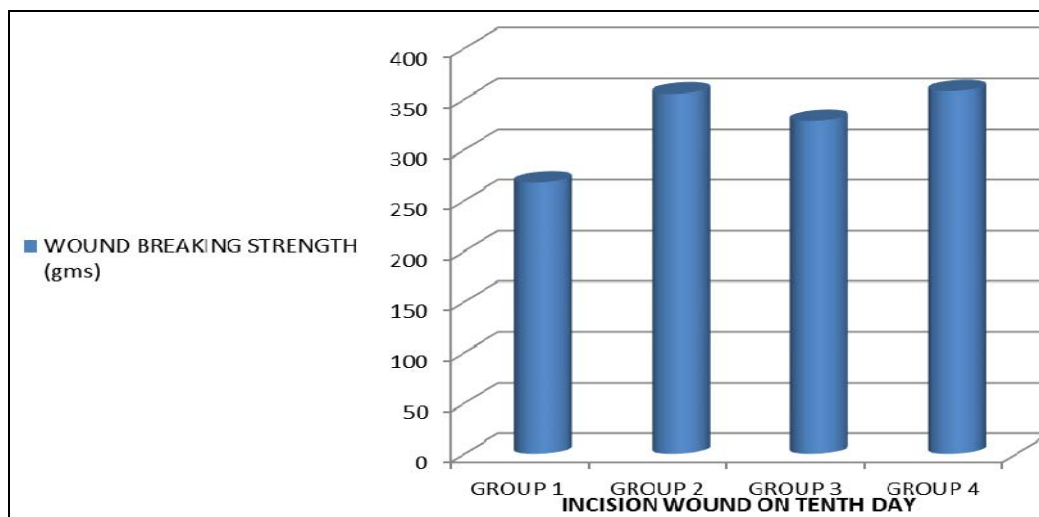


Fig 6: Effect of AEES on breaking strength (g) in incision wound model

4. Discussion

Present study has undertaken to estimate the wound healing activity of *Emilia sonchifolia* in experimentally produced wounds in rats. Literature studies reveals the wound healing activity. Preliminary phytochemical studies reveals the presence of carbohydrates, glucosides, saponins and flavanoids.

The skin irritation study was performed on the rats proved that the aqueous extract of *Emilia sonchifolia* does not produce any type of skin irritation. This is a navigating property for wound healing of *Emilia sonchifolia*.

Inn excision wound method group 3and group 4 which received aqueous extract *Emilia sonchifolia* showed faster wound healing acting than the control group. The epitheliation process and wound contraction period also showed marked decrease compared to control group. These effect activity can be attributed to flavonoid content of the present drug. Flavonoids reduce lipid peroxidation and prevents cell necrosis and improved vascularis.

In incision model, the wound breaking strength was increased than control but less than that of standard drug. *Emilia sonchifolia* was potent as compared with cipladine in excision

and incision wound models.

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