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Original Research Article.

Study of Wound Healing Activity of Parthenium Hysterophorus by Excision Wound Model in Rats

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ABSTRACT

Fresh Parthenium hysterophorus was collected from the nearby area and was authenticated by local botanist of Science College. Aerial parts were shade dried and powdered in the department of pharmacology. The powder (Aerial parts) was macerated for 24 hours in 70 %v/v ethanol. The hydro-alcoholic extracts was obtained by percolation using 70 % v/v ethanol as a solvent. Percolated solution was again shade dried and extract was obtained for topical application *Parthenium hysterophorus* extract was mixed with simple ointment as mention below.

In excision wound model, 5% ointment of PH extract showed reduction in wound area on 4th day which was not significant but 8th day onwards reduction in wound area was very significant ($p < 0.01$) as compare to control. Whereas 10% ointment of PH extract and standard both showed reduction in wound area on 4th day onward which was highly significant ($p < 0.001$) as compare to control.

Keywords: Atrophine, Atropa Ephedra, Vulgaris, Aswagandha.

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INTRODUCTION

Plants have been a main source of large numbers of drugs being used today. The well-known amongst such drugs are morphine from Papaver somniferum which is used to alleviate pain; aswagandha from Withania somnifera to reduce inflammation; ephedrine from Ephedra vulgaris for treatment of bronchial asthma; atrophine from Atropa belladonna for treatment of cancer etc. Plants play an essential role in the health care needs for the treatment of diseases and to improve the immunological response against pathology. Parthenium hysterophorus is an aggressive ubiquitous annual herbaceous weed with no economic importance unravelled till now. It is commonly known as carrot grass, bitter weed, star weed, white top, wild feverfew, the ''Scourge of India'' and congress grass. Parthenium hysterophorus is a prolific weed belonging to Asteraceae family. The decoction of P. hysterophorus has been used in traditional medicine to treat fever, diarrhoea, neurologic disorders, urinary tract infections, dysentery, malaria and as emmenagogue.[1] Parthenium hysterophorus has been found to be pharmacologically active as analgesic in muscular rheumatism, therapeutic for neuralgia and as vermifuge.[2]

Parthenin, the major constituent of the plant, exhibits significant medicinal attributes including anticancer property.[3] The flowers showed significant anti tumour activity and parthenin exhibited cytotoxic properties against T cell leukaemia, HL-60 and Hela cancer cell lines.[4] P. hysterophorus has hypoglycaemic activity against alloxan-induced diabetic rats. So, flower extract of this weed can be used for developing drug for diabetes mellitus.[5]

Till now very less work has been done regarding wound healing activity of Parthenium hysterophorus**.** Hence this study will be conducted to evaluate these properties of Parthenium hysterophorus.

MATERIALS AND METHODS

Animals

Healthy adult Wister rats of either sex weighing 150 – 250 gm were used.

Plant

Fresh Parthenium hysterophorus was collected from the nearby area and was authenticated by local botanist of Science College. Aerial parts were shade dried and powdered in the department of pharmacology.

PREPARATION OF EXTRACT

The powder (Aerial parts) was macerated for 24 hours in 70 %v/v ethanol. The hydro-alcoholic extracts was obtained by percolation using 70 % v/v ethanol as a solvent. Percolated solution was again shade dried and extract was obtained.

PREPARATION OF DRUG FORMULATION

For topical application *Parthenium hysterophorus* extract was mixed with simple ointment as mention below.

PREPARATION OF OINTMENT

Simple Ointment (100gm IP)-

Soft Paraffin: 85 gm
Hard Paraffin: 10 gm
Lanolin/wool fat: 5 gm

5% (w/w) Ointment

Simple ointment: 95gm
PH extract: 5 gm

10% (w/w) Ointment

Simple ointment: 90gm
PH extract extract: 10 gm

DRUGS, CHEMICALS AND INSTRUMENTS

a) Drugs

- Pentobarbitone- (Loba Cheme IndoAustranal Co, Mumbai)
- Soframycin 30 gm (Aventis Pharma Limited, Goa)

b) Instruments-

- Percolator- Borosil (Alka Scientific Co. Nagpur)

ETHICAL CLEARANCE

Ethical clearance was taken from Institutional Animal Ethics Committee.

METHOD FOR EVALUATING WOUND HEALING ACTIVITY

a) Excision Wound Model[6, 7]

The albino Wistar rats of either sex were divided into 4 groups, 6 animals in each group (total 24 animals)

Group 1: Control-simple ointment locally once daily
Group 2: PH extract (5% ointment) locally once daily
Group 3: PH extract (10% ointment) locally once daily
Group 4: Standard drug Soframycin ointment locally once daily

The animals were anesthetized by using pentobarbitone (30 mg/kg *i.p*). An impression was made on the dorsal thoracic region 1 cm away from vertebral column and 5 cm away from ear on the anaesthetized rat. The particular skin area was shaved one day prior to the experiment. The skin of impressed area was excised to the full thickness to obtain a wound area of about 500 mm^2. Haemostasis was achieved by blotting the wound with cotton swab soaked in normal saline. The wound contraction was studied by tracing the raw wound area on the subsequent day 1, 4, 8, 12, 16, 18 and 21 on graph paper. Scar area and time for complete epithelisation were also measured. The percentage of wound contraction was recorded.

Wound contraction =

$$\frac{\text{Area on day zero - Area on day of measurement x 100}}{\text{Area on day zero}}$$

All the results were expressed as Mean ± Standard Deviation (SD). The differences between experimental groups were compared by one-way Analysis of Variance (ANOVA) followed by test. The results were considered statistically significant when *p < 0.05, **p< 0.01-very significant, ***p< 0.001-Highly significant as compare to control.

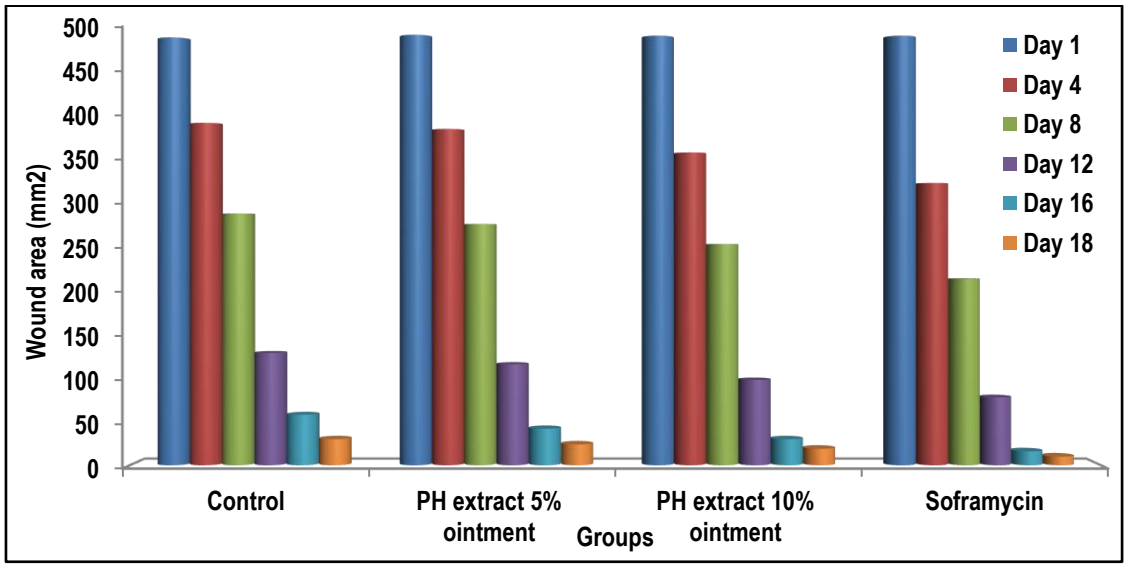
RESULTS

In excision wound model, 5% ointment of PH extract showed reduction in wound area on 4th day which was not significant but 8th day onwards reduction in wound area was very significant (p <0.01) as compare to control. Whereas 10% ointment of PH extract and standard both showed reduction in wound area on 4th day onward which was highly significant (p <0.001) as compare to control. (Table 1, graph A, B) (Figure C, D, E and F)

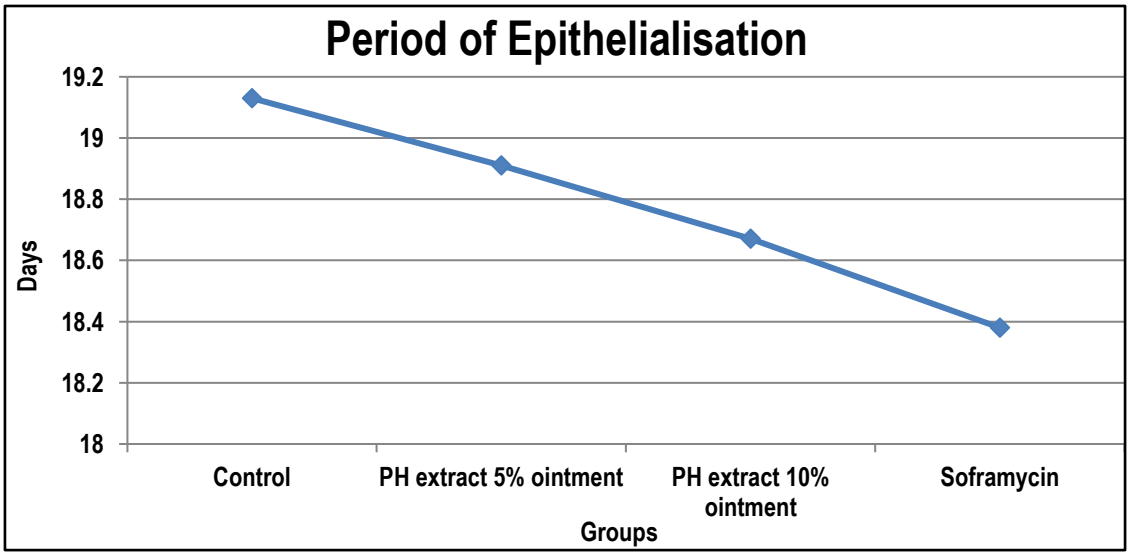
Table 1: Wound healing activity of PH extract by Excision wound model on rats.

Groups	Post wounding days wound area (mm^2) mean ± SD (Percentage of wound contraction)						Period of epithelialisation (days)
	Day 1	Day 4	Day 8	Day 12	Day 16	Day 18	
1-Control Simple ointment	491± 4.321 (0.0)	393 ± 7.668 (19.95)	289 ± 7.737 (14.41)	129 ± 5.573 (73.72)	58± 5.154 (88.18)	30 ± 4.033 (94.09)	19.13
2- PH extract 5% ointment	494± 2.876 (0.0)	386 ± 7.884 (21.86)	277 ±6.470** (43.92)	116 ± 9.368* (76.51)	42 ± 9.948** (91.49)	24 ± 3.391** (95.14)	18.91
3-PH extract 10% ointment	493± 3.654 (0.0)	359 ± 15.068*** (27.18)	254 ± 15.567*** (48.47)	98 ± 12.596*** (80.12)	30 ± 12.533*** (93.91)	19 ± 4.708** (96.41)	18.67
4-Standard Soframycin	493± 3.654 (0.0)	324 ± 25.732*** (34.27)	214 ± 30.024*** (56.59)	78 ± 8.958*** (84.17)	16 ± 17.557*** (96.75)	10± 3.656*** (97.91)	18.38

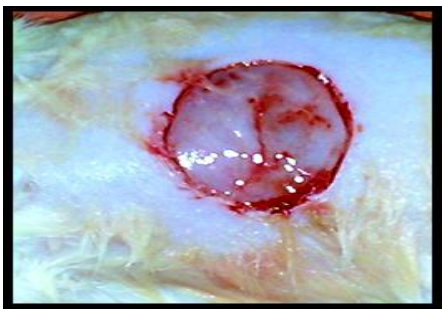
Number of animals n=6; PH- Parthenium Hysterophorus; Results are expressed in Mean ± SD; *P<0.05-significant,
** P <0.01- very significant ***P < 0.001- Highly significant compare to control



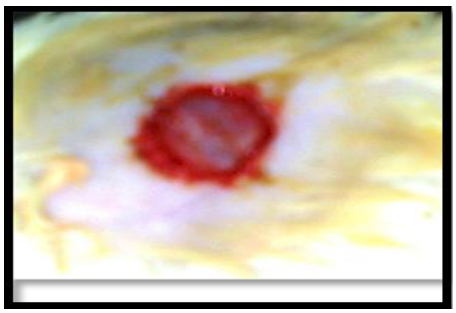
Graph A: Wound healing activity of *Parthenium Hysterophorus*extract on rats by Excision wound model.



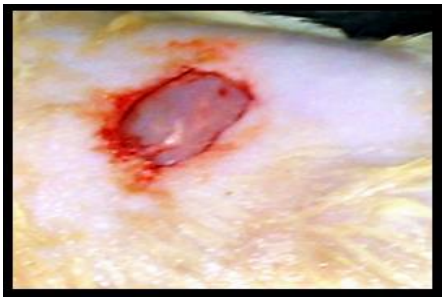
Graph B: Wound healing activity of *Parthenium Hysterophorus* extract on rats by Excision wound model showing period of epithelialisation.



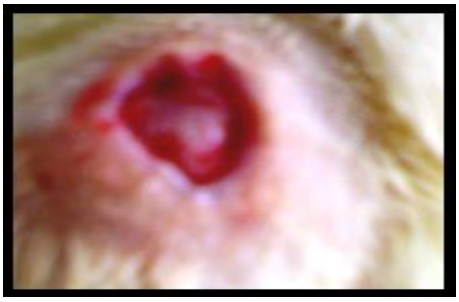
A-Control



B- PH extract 5% ointment

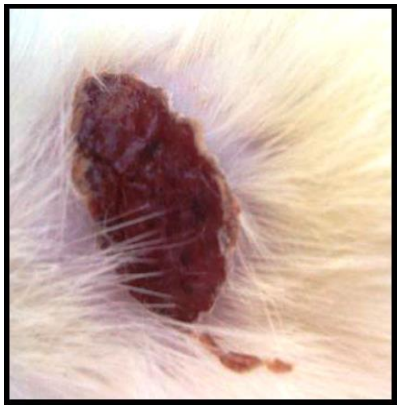


C- PH extract 10% ointment

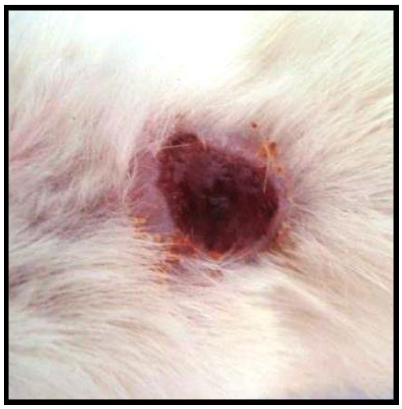


D-Standard (Soframycin)

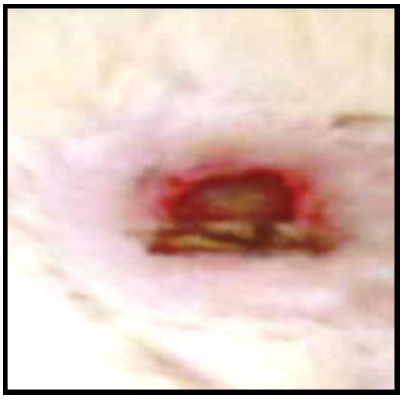
Fig C: Excision wound on first day.



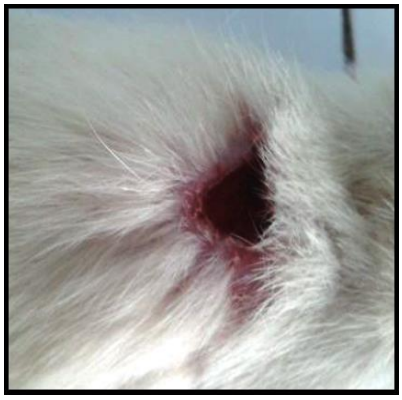
A-Control



B-5% ointment

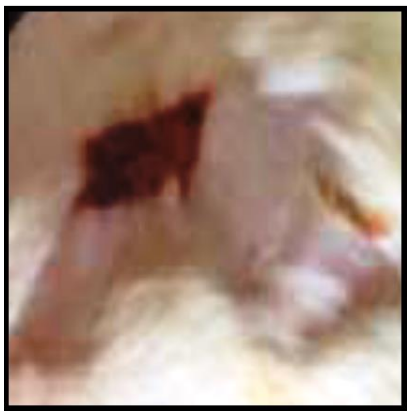


C-10% ointment

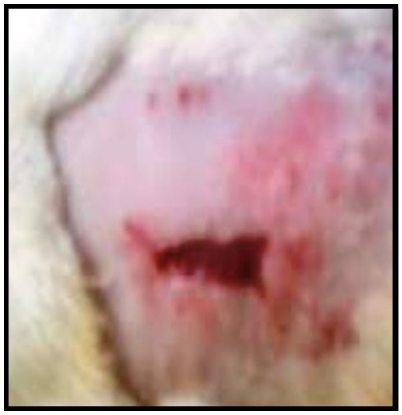


D-Standard (Soframycin)

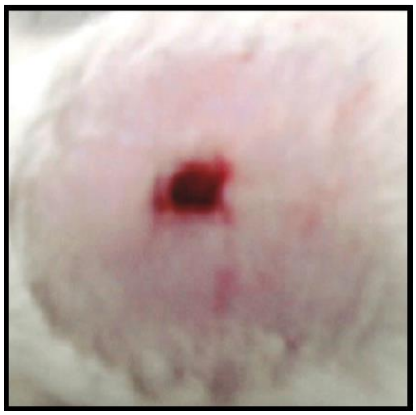
Fig D: Excision wound on 8thday.



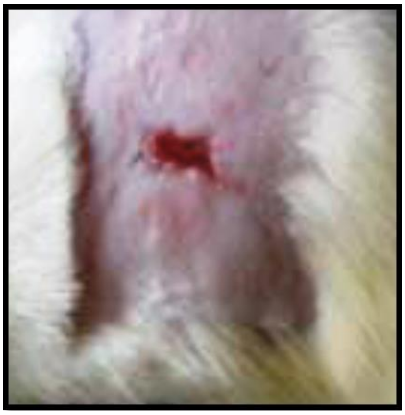
A-Control



B-5% ointment

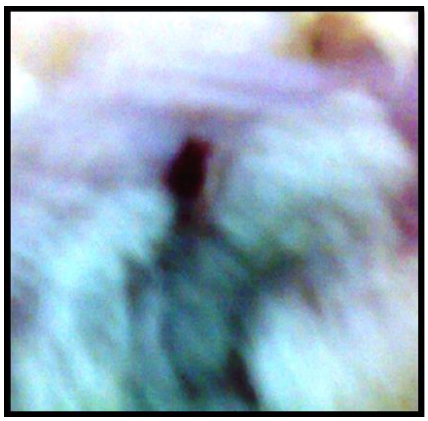


C-10% ointment

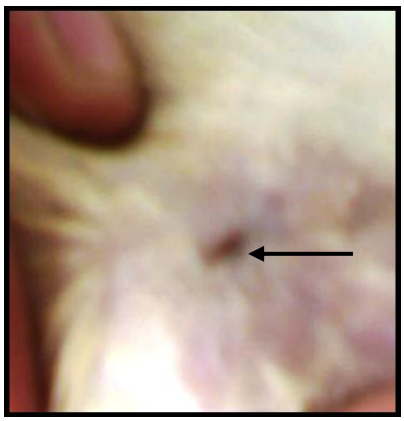


D-Standard (Soframycin)

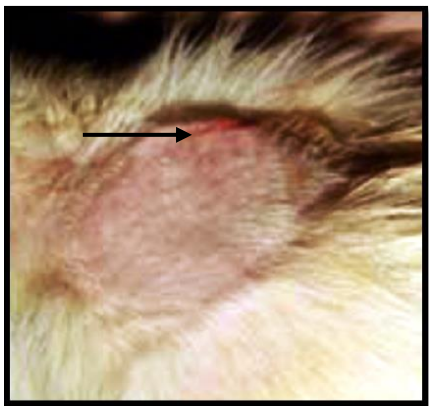
Fig E: Excision wound on 12thday.



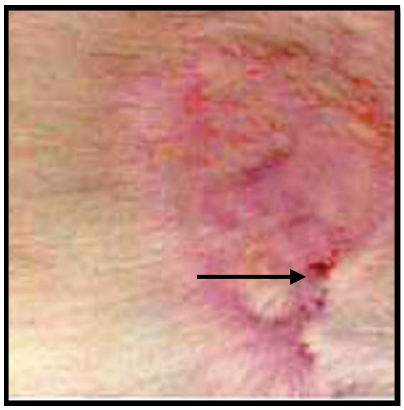
A-Control



B-5% ointment



C-10% ointment



D-Standard (Soframycin)

Fig F: Excision wound on 16thday

DISCUSSION

Wound healing is a natural process of regenerating dermal and epidermal tissues. Whenever there is a wound, a set of overlapping events takes place to repair the damage.[8] These processes have been categorized into phases which include the inflammatory, proliferative and the remodeling phases.[9] In the inflammatory phase, bacteria and debris are phagocytosed and removed and cytokines and mediators are released that cause the migration and division of cells involved in the proliferative phase. Angiogenesis, collagen deposition, granulation tissue formation, epithelialization, and wound contraction occur in the proliferative phase.[10] During epithelialization, the epithelial cells crawl across the wound bed to cover it.[11] The wound is eventually closed by a combination of all these and by the process of wound contracture. During wound contraction, the wound is made smaller by the action of myofibroblasts, which establish a grip on the wound edges and contract themselves using a mechanism similar to that in smooth muscle cells. In the maturation and remodeling phase, collagen is remodeled and realigned along tension lines and cells that are no longer needed are removed by apoptosis.

The granulation tissue of the wound is primarily composed of fibroblast, collagen, edema, and small new blood vessels. The undifferentiated mesenchymal cells of the wound margin modulate themselves into fibroblast, which start migrating into the wound gap along with the fibrin strands. The collagen composed of amino acid (hydroxyproline) is the major component of extra cellular tissue, which gives strength and support. Breakdown of collagen liberates free hydroxyproline and its peptides; measurement of the hydroxyproline could be used as an index for collagen turnover.

Lipid peroxidation is an important process of several types of injuries like burn, inflicted wound and skin ulcers. A drug that inhibits lipid peroxidation is believed to increase the viability of collagen fibrils, increasing the strength of collagen fibers by an increase in circulation, thereby preventing the cell damage and promoting the DNA synthesis. Antioxidants such as metronidazole, vitamin C, vitamin E have been shown to promote wound contraction and epithelization.[12]

In excision wound model, 5% ointment of Parthenium hysterophorus extract showed significant ($p < 0.01$) reduction in wound area on 8th day onwards as compare to control. Whereas 10% ointment of Parthenium hysterophorus extract and standard drug soframycin showed reduction in wound area on 4th day onward which was highly significant ($p < 0.001$) as compare to control.

Kuhn andWinston, (2007)[13]; Zhou et al. (2011)[14], suggest that the antioxidant property of the Parthenium hysterophorus extract conferred upon it by the presence of high Apigenin, Canin[15] may also be responsible to the prohealing action of the extract

Literature survey has revealed that Apigenin Canin promote wound healing activity through several mechanisms that include chelation of free radicals; antioxidant, antimicrobial properties.[14, 15]

Lipid peroxidation, results in cellular membrane damage which leads to swelling and cell death. The free radicals attract the different inflammatory mediators that are responsible for the general inflammatory response and tissue damage. During injury there is an increase in the consumption of the endogenous anti-oxidants that bring about a decrease the amount of anti-oxidants. Flavonoids may contribute an additive effect to the endogenous

anti-oxidants and to inhibit the eicosanoid biosynthesis therefore decreasing the formation of the inflammatory metabolites which is responsible for its anti-inflammatory property. Hence by virtue of their free radical scavenging, antioxidant and anti-inflammatory properties, flavonoids may help in healing of wounds.[16]

Thus it can be suggested that phytochemical constituents present in Parthenium hysterophorussuch as Apigenin, Canin, flavonoids etc may be responsible for wound healing activity.

From our and other study it is very clear that Parthenium hysterophorus extract has wound healing activity.

SUMMARY AND CONCLUSION

Parthenium hysterophorus extract as 5% and 10% ointments showed significant reduction in wound area and increase in tensile strength as that of standard drug (soframycin). It can be due to antioxidant property of the Parthenium hysterophorus conferred upon it by the presence of high amounts of apigenin may also be responsible to the prohealing action of the extract**.** Thus it can be suggested that phytochemical constituents present in Parthenium hysterophorussuch as Luteolin, Parthenolide, Pathenolide, Reynosin, Santamarin, Santin, Apigeninetc may be responsible for it wound healing activity.

Further elaborative work is necessary for the better understanding of the mechanism of wound healing activity of Parthenium hysterophorus. For clinical use detailed clinical studies are required in future in humans.

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