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Research Article

EVALUATION OF WOUND HEALING POTENTIAL OF AQUEOUS AND ETHANOLIC EXTRACTS OF *TRIDAX PROCUMBENS* LINN. IN WISTAR RATS

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ABSTRACT

Tridax procumbens Linn. is a medicinal plant which is used from time immemorial for various disorders especially cuts, wounds and burns. The objective of the study presented here was to verify the traditional claims by using aqueous and ethanolic extracts of the whole plant of *Tridax procumbens* Linn. for its wound healing property by using animal models. For both excision and incision wound model the animals were divided into four groups of control and treatment. In incision wounds, tensile strength of the wound in the drug treated animals were increased much more significantly as compared with control group animals. In excision wound model the rate of wound contraction was assessed as healing parameter at every 3rd day. On day 15th biochemical tissue markers like Hydroxyproline, Collagen and Hexosamine were determined from excised tissue and they were significantly increased in plant extract treated groups. Statistically significant reduction in the wound area was found in the treated groups compared to control untreated group ($P<0.05$). It has been studied previously that stage of wound healing involves acute inflammatory phase followed by the synthesis of collagen and other extra cellular macromolecules, which is later removed to form a scar. In the present experiment, histopathological observations showed increase in granulation and rapid collagen turnover.

Keywords: *Tridax procumbens* Linn., Excision and incision wound, Hydroxyproline.

INTRODUCTION

Wounds are physical injuries that result in an opening or break of the skin.[1]It is a process that is fundamentally a connective tissue response. Initial stage of this process involves an acute inflammatory phase followed by synthesis of collagen and other extracellular macromolecules that helps in the formation of a scar. This intricate process that is initiated in response to an injury restores the function and integrity of damaged tissues.[2] This dynamic process is briefly divided into three overlapping phases "inflammation, proliferation and remodeling".[3] There are various natural agents, which assist in wound healing process and one of the folkloric plant that is amply available was selected for the present experimentation. *Tridax* is a week straggling herb about 12-24cm long with few leaves 6-8cm long. They are simple, opposite, exstipulate and ovate. Two types of flowers such as rayflorets and disk-florets and very long slender solitary peduncle is the characteristic of this plant.[4] In English *Tridax procumbens* Linn. is popularly called as 'coat buttons' because of appearance of flowers. In Sanskrit the plant is commonly known as 'Ghamara' which has been extensively used in Ayurvedic system of medicine for various ailments. Many ayurvedic practitioners use this plant as medicines in liver disorders[5]. The plant is native of tropical America, tropical Africa, Asia, Australia and India. It is a wild herb distributed throughout India. Coat buttons are found along railroads, riverbanks, meadows, roadsides, waste grounds, dikes and dunes. As per traditional use and pharmacological consideration it is well known for hepatoprotective activity,[6] Immunomodulatory activity,[7] Wound Healing activity,[8] Antidiabetic Activity,[9,10] .Antimicrobial Activity[11], [12]and are also promising mosquito repellents [13]. Moreover, leaves of *Tridax* are good hair growth promoters and have ability to prevent falling of hairs. [14] This plant was also used for bronchial catarrh, dysentery, diarrhoea in the West Africa and as a remedy against conjunctivitis. [14,]

MATERIALS AND METHODS

Drugs and Chemicals: standard drug Cipladine was obtained from Cipla Pvt Ltd. Ketamine and xylazine were procured from Aquafine injecta Pvt Ltd. and Indian Immunologicals Ltd. respectively. All other chemicals used for the study were of analytical grade.

Plant material

Whole plant of the *Tridax procumbens* (TP) was collected from Kem village of Solapur district of Maharashtra in the December, 2010. The plant material was authenticated from Botanical Survey of India, Pune and the voucher specimen was submitted to APT Research Foundation, Pune

Preparation of Extract

Shade dried Plant material was extracted by using Soxhlet apparatus. Aqueous and ethanolic extracts were made with the respective solvents. Each was kept for 24 hrs and then concentrated under vacuum to a thick paste which were further used for application to the rats.

Animals

Adult Wistar rats (180-200gms) of both sexes were procured from National Toxicology Centre, Pune. Total 24 animals were divided into four groups (control, standard, aqueous extract treated and ethanolic extract treated) with 6 animals in each group. Animals were housed under standard environmental conditions of temperature (23^0C) and 12 hours light and dark cycle. All the animals were provided with food and water *ad libitum*. Study protocol was approved by Institutional Animal Ethical Committee and conducted according to the guidelines of CPCSEA.

Acute dermal toxicity

Swiss albino female mice of 18-22g weight and age of 90 days were used to determine the dermal toxicity of test extracts. The toxicological study was carried out to determine the therapeutic dose of the aqueous and ethanol extracts as per the OECD guidelines. Testing of the ethanolic extract and the aqueous extract were done by applying the aqueous and ethanolic extracts of the highest concentrations on the shaved dorsal sides of the rats. It was observed that the dose was safe and lower dose was considered for further study.[15]

Animal testing

For the *in vivo* wound experiment incision and excision wound models were used. Test extracts were prepared and diluted in double distilled water and applied at a dose of 200 mg/kg. Test extract was applied

topically on the wounded site immediately after creating circular wounds by a surgical blade. The control group of animals was not treated with any drug and wounds were kept open[16]. Whereas the standard drug treated group of animals were applied with reference drug cipladine.[17]

Linear incision wound model

All the animals were anaesthetized with 1:1 ketamine hydrochloride and xylazine and the back hair of the rats were shaved by using a shaving machine and impression was made on dorsal region 1cm away from vertebral column and 5cm away from ear. Linear paravertebral incision of 5cm long was made through the full thickness of the skin. Wounds were closed with interrupted sutures, which were removed on the 10th day after wound creation. Incision wounds were treated with the extracts daily for 14 days. The Wounds in control group of animals were kept open and was allowed to heal naturally. On 14th day after formation of wound the breaking strength of the wound (in kilograms) and was measured by using Tensiometer.[18] A portion of the incised skin was sent for histopathological examination for assessing re-epithelization and collagen formation.[19]

Excision wound model

The animals were anaesthetized by injecting intramuscularly ketamine hydrochloride and xylazine in 1:1 concentration. The dorsal fur of the animals was shaved with shaving machine. Impression was made on dorsal region and area of the wound to be created was marked on the back of the animals by picric acid using circular stainless stencil. Using toothed forceps and pointed scissors circular excision wound of 300 to 400 mm^2 were created to full thickness along the markings.[20] Wound areas were measured by tracing the wound on transparency sheet with permanent marker by using millimeter based graph paper on days 0, 3rd, 6th, 9th, 12th and 15th for all groups.[21, 22]

Preparation of test samples for bioassay

The extracts, the reference drug and the vehicle were applied topically once a day till the 15th day. At an interval of every three days, changes in wound area were monitored.[23] and also the wound area was evaluated by using graph paper. Percentage of the reduction in wounded area was calculated from wound contraction. Histopathological examination[24] and biochemical parameters were carried out by using tissue specimen isolated from the healed skin of each groups of rat.

Histopathology

10% formalin was used to fix the tissue and was embedded in paraffin wax. Serial sections of paraffin embedded tissues were made. Staining was done by using Haematoxylin and eosin, which were examined by light microscope. Congestion, edema, polymorpho nuclear leukocytes (PMNL), mononuclear cells, fibroblasts and vascularization were qualitatively evaluated as well as ulceration, necrosis and epithelialization were examined in the skin tissues.

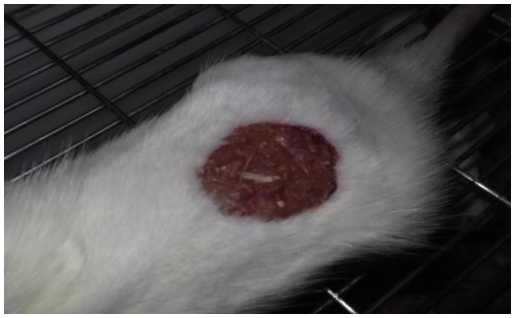
Biochemical parameters

Circular wound area was excised and evaluated for various biochemical parameters at the end of the study. Especially Collagen content, Hydroxyproline[25] and Hexosamine[26] was estimated for evaluating the healing properties of both the extracts of *Tridax procumbens* Linn.

Statistical analysis

Results obtained from the two wound healing models have been expressed as Mean ± SD and were compared with the corresponding control group by one way ANOVA test for assessing statistical significance.[27]

RESULTS



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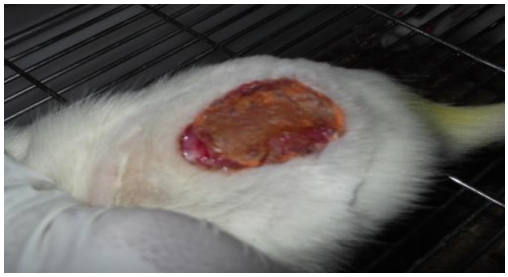

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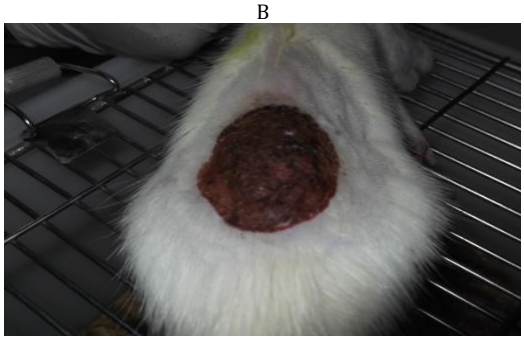

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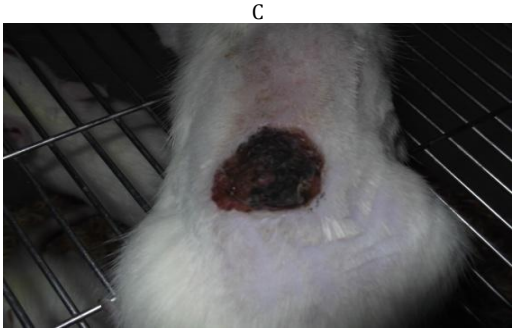

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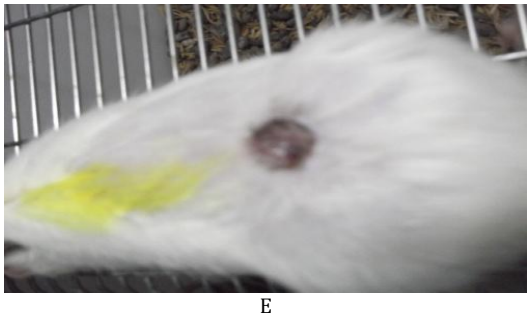

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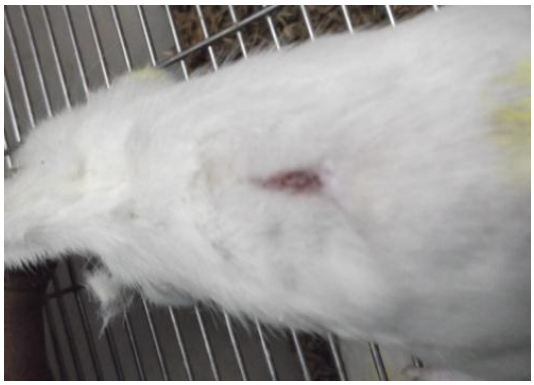

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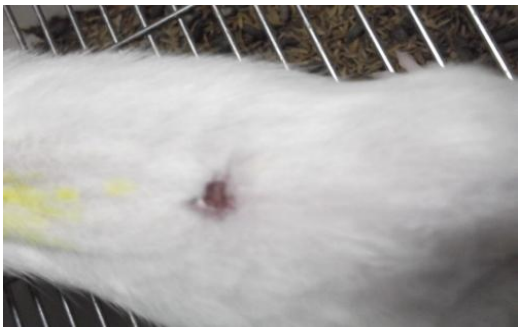

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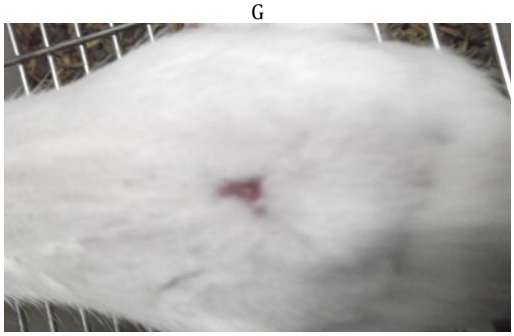

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Fig 1: A, B, C and D are the wound of control, standard, Aqueous extract and ethanolic extract at 0 day and E, F, G and H are the wound of control, standard, Aqueous extract and ethanolic extract at 15th day respectively.

Incision wound model.

In the linear incision wound model there was a significant increase in the tensile strength of the wounds when treated with both the aqueous and ethanolic extracts of TP. The tensile strength required to disrupt the wound was found to be 1.9 kg and 2.0 kg as compared to the vehicle control which was 1.55 kg. Similarly, the standard drug treated animals needed 1.9 kg to tear out the wound. The results were statistically significant at $P<0.05$. (Fig 2)

Histopathological observations.

Treatment of rat wounds with plant extract of *Tridax procumbens* Linn. and standard drug treated animals led to reduced polymorphonuclear leukocytes (PMNLs), congestion, oedema, mononuclear leukocyte infiltration and necrosis. *Tridax procumbens* Linn. treated animals were found to have mild vascular proliferation and reduction of accessory skin structures. Along with these, considerable increase in the dermal collagen content was evident from the histopathological observation. On the contrary, in disease control group focal dermal fibrosis, brownish pigments in macrophages were observed. (Fig 3)

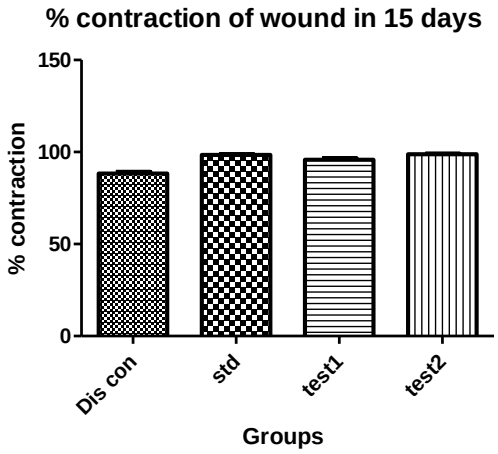
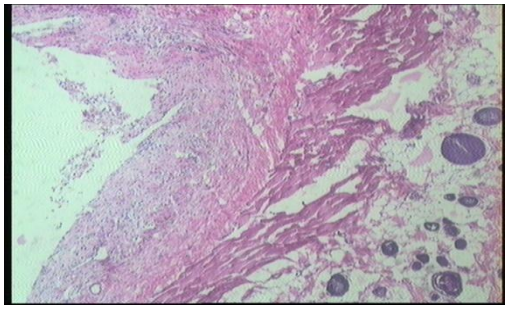


Fig 2 : Tensile strength of incision wound



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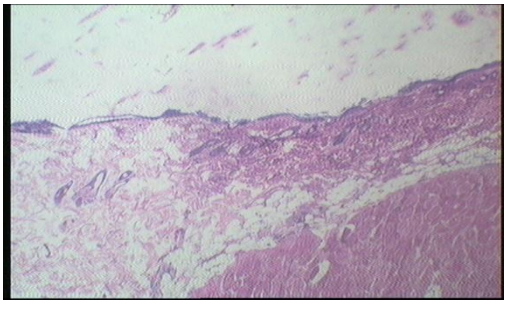

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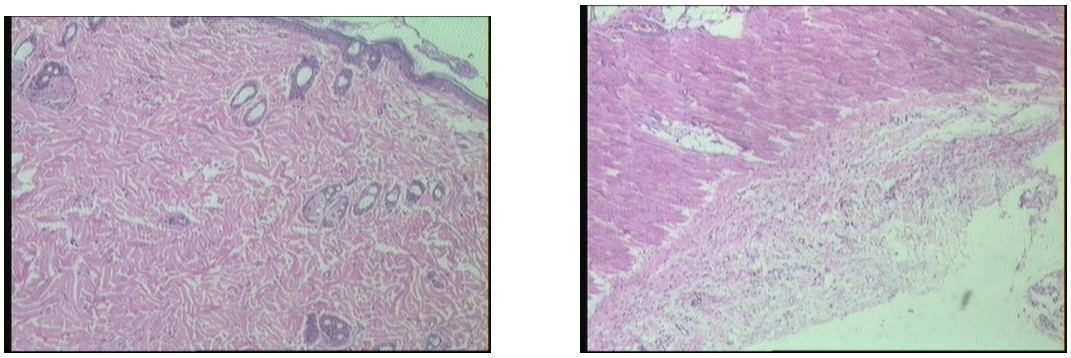

C **D**

Fig 3: A, B, C and D are the histopathology of the skin tissue of control, standard, Aqueous extract and ethanolic extract treated rats at 15th day.

Excision wound model

In the excision wound model, the wound size gradually decreased at an interval of 15 days. Animals treated with aqueous extract of TP showed 17.0±7.9mm^2 wound area while the ethanolic extract treated group showed 4.5±2.4 mm^2 wound area where as the area of wound was found to be 45±7.9 mm^2 and 6.3±3.8 mm^2 in untreated control group and the standard drug respectively. The results were found to be statistically significant at P<0.05 when compared with untreated vehicle control group. (Table 1)

Table 1: Effect of *Tridax procumbens* in excision wound contraction

GROUP	0 DAY	3rd DAY	6th DAY	9th DAY	12th DAY	15th DAY
CONTROL	388.5±7.06	325.0±5.8	292.0±5.5	141.0±8.0	60.5±6.1	45.3±7.9
STANDARD CIPLADINE	405.3±7.9	236.7±25.4***	149.8±9.8***	61.8±6.4***	23.3±3.6***	6.3±3.8***
AQUEOUS EXTRACT	404.8±6.5	259.7±45.2***	187.3±5.9***	75.2±7.5***	53.5±6.2***	17.0±7.9***
ETHANOLIC EXTRACT	398.0±8.0	202.3±14.7***	90.3±5.7***	38.2±2.9***	18.7±2.5***	4.5±2.4***

Data: Mean± SD *** P<0.05 when compared with control group.

Effect of *Tridax procumbens* on Hydroxyproline, Collagen and Hexosamine content.

There was a significant increase in the hydroxyproline content that is 80.85 ± 4.10 and 86.03 ± 4.19µg/gm in aqueous and ethanolic extract treated group respectively which was much more higher than disease control and standard drug treated group which showed the values of 41.88 ± 3.82 and 67.90 ± 4.84µg/gm . Generally an increase in hydroxyproline content is ultimately responsible for increase in collagen levels. In the present study control and standard drug treated animals showed much lesser collagen content which was 312.45±7.33and 506.53±8.20µg/gm as compared to aqueous and ethanolic extracts treated groups which showed 603.14±30.61 and 641.81±31.27µg/gm concentration of collagen respectively. For healing property the hexosamine content was evaluated in the animal tissues which showed 24.75±1.48 and 27.28±2.32mg/gm in the aqueous and the ethanolic extract treated group while 8.7 and 20.7 mg/gm in disease control and standard drug treated group respectively. The values were statistically significant at P<0.05 when compared to untreated vehicle control group. (Table 2)

Table 2: Effect of *Tridax procumbens* Linn. on biochemical parameters of wound healing.

GROUP	HYDROXYPROLINE (µg/gm)	COLLAGEN (µg/gm)	HEXOSAMINE (mg/gm)
CONTROL	41.88±3.82	312.45±7.33	8.77±0.83
STANDARD	67.90±4.84	506.53±8.20	20.73±1.67
AQUEOUS EXTRACT	80.85±4.10	603.14±30.61	24.75±1.48
ETHANOLIC EXTRACT	86.03±4.19	641.81±31.27	27.28±2.32

Data: Mean± SD *** P<0.05 when compared with control group.

DISCUSSION

In the present study, the aqueous and ethanolic extracts of the whole plant of *Tridax procumbens Linn. an* indigenous medicinal plant of Asia was evaluated for the wound healing activity. This plant is widely distributed and it's each and every part having noble pharmacological activity. [28]

Wound healing involves a complex interaction between epidermal and dermal cells, the extra cellular matrix, controlled angiogenesis and plasma-derived proteins all coordinated by an array of cytokines and growth factors [29]. Aqueous extract was also effective in increasing wound contraction but to a lesser degree than ethanolic extract. In the excision wound model we observed that the aqueous and ethanolic extracts of TP showed 95.79% and 98.86% wound contraction whereas, the standard drug, cipladine treated group and untreated control group showed 98.45% and 88.33% wound contraction on the 15 days study period.

In the linear incision wound model, we measured the tensile strength of the incision wound. In this study we found that the topical application of ethanolic extract of the plant showed significantly higher tensile strength than the aqueous extracts, where as standard and disease control groups showed much lesser tensile strength needed to break the wound than the extracts treated groups.

It was also found in another study that Whole plant of *Tridax* has antimicrobial activity on various species of bacteria. Owing

to this property it is used to provide protection against human dermal infection and it might facilitate faster wound healing[30].

In the present experiment, the plant increases not only granulation and hexosamine formation but also, showed significant increase in hydroxyproline content of the granulation tissue of the excision wound which indicated rapid collagen formation. Both the extracts also showed an increase in hexosamine content which leads to rapid healing of wounds[31]. Considering the obtained results we can assume that the plant of *Tridax procumbens* Linn. might become a useful component for healing the wounds. Thus, further efforts will be put forth towards emphasizing its active components responsible for its wound healing potential.

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