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EVALUATION OF WOUND HEALING POTENTIAL OF DIFFERENT TOPICAL FORMULATIONS OF METHANOLIC LEAF EXTRACTS OF *TRIDAX PROCUMBENS* L. IN RATS

Pharmacology

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ABSTRACT

Tridax procumbens L. (TP) is a common weed that grows in the rice fields of India. Traditionally the leaves of *Tridax procumbens has* been used for wound healing. However, in experimental studies, equivocal pro and anti-healing action of *Tridax procumbens* has been demonstrated. The present study evaluates the effect of topical ointment different formulations like Ointments (5% & 10%), Creams (5% & 10%) and Gels (5% &10%) of the Methanolic leaf extracts of *Tridax procumbens*(METP) using excision wound model in rats. Excision wounds (2.5 cm, i.d.) were inflicted on depilated back of rats. Formulations of METP were applied twice daily for 21 days on the dermal wound. The parameters observed were Wound area, re-epithelization, vascularity, fibroblast number, collagen content. The METP of Ointment, Creams, Gels (10%) and Silver sulphadiazine (1%) Showed statistically very significant (P>0.01) when compared to control group at 21st day. Taken together, the results imply that METP possesses dose dependent pro-healing potential. The Order of potency Silver sulphadiazine> METP Ointment base> METP Cream base > METP Gel base.

KEYWORDS:

Collagen content, Fibroblast number, *Tridax procumbens,* Wound area.

Introduction:

The pathologic condition of dermal wound commonly defined as any break in the integrity of the skin. It is related with high degree of morbidity due to blood loss, pain, edema, inflammation and loss of functionality. The cutaneous wounds are characterized by migration and proliferation of fibroblasts, endothelial and epithelial cells, and deposition of connective tissue, angiogenesis, re-epithelization and finally contraction of wound[1].

Healing of wounds is needed for restoration of disrupted anatomical continuity and disturbed functional state. Impaired healing of open wounds is one of the troublesome complications that have been recognized for many years. It is debatable, whether systemic drugs can hasten healing in a nutritionally and endocrinally normal individual. In such cases, the basic principles of wound healing that include minimizing tissue damage, debriding nonviable tissue, maximizing tissue perfusion and oxygenation, proper nutrition, and a moist wound healing environment prove to be most useful. Thus, a drug that can enhance vascularization, reepithelization, and collagenation, when applied topically, should prove ideal[2].

Traditional medicinal system of Indian is fundamentally based on Ayurveda. According to Ayurveda different plant extracts had significantly contributed for remedial effects on mankind. Till today plant materials serves as the potential sources of drugs[3] *Tridax procumbens* L. belongs to the family of Asteraceae and commonly known as 'Gaddi chamanthi' in Telugu, in Ayurvedic as Jayanthi, in Sidda/Tamil as Vettukkaaya-thalai, in Folk as Akala kohadi and in English as Coat buttons/Mexican Daisy, because of the appearance of its flowers and is an ethno botanically important medicinal plant. The plant has been considered as a gregarious weed, distributed throughout the tropics and sub tropics. Traditionally in India, *Tridax procumbens* leaves have been used as one of the most popular remedy for dermal wounds. In excision wound model, systemic administration (intrap eritoneal) of juice from leaves of *Tridax procumbens* has been implicated with both pro and antihealing properties[4]. Earlier workers have reported that it possesses antidiabetic, anti-bacterial, antipla smodial, antihepatotoxic, anti-oxidant, antimicrobial, immuno modulatory and anti-cancerous[5-11] properties. Its flowers and leaves possess antiseptic, insecticidal and parasiticidal properties [12,13]. In order to clearly establish its activity, the present study was focused on Wound healing activity of Methanolic extract of *Tridax procumbens* with different formulations.

Material and Methods:

Plant material, extract and formulation preparation:

Leaves of *Tridax procumbens* L. were collected from different localities of Bangalore and its nearby areas and washed thoroughly with distilled water. The cleaned plant parts are then allowed for the complete shade drying and then made to fine powder with a mechanical grinder and stored in an airtight container. A powdered plant parts were extracted successfully with the methanol by using Soxhlet apparatus. The extraction was carried out for 24 hours at room temperature with mild shaking. The extracts were filtered and concentrated at 45^0 C using rotary vacuum evaporator. The obtained extracts were vacuum dried and made formulations like ointment base (5% & 10%), cream base (5% & 10%) and gel base (5% & 10%).

Ointment base formulation

SL. No.	Base	Quantity
1	White Soft paraffin	Q.S 100%

Cream base formulation: Oil in water type cream

SL.No.	Base	Quantity
1	Stearyl alcohol	15%
2.	Bees wax	8%
3.	Sorbitol Monooleate	1.23%

Gel Base formulation

SL.No.	Base	Quantity
1.	PEG 4000	5%
2.	PEG 400	5%
3.	Distilled water	Q.S

Animals:

Wister albino rats of weighing about 150- 200 gm were employed for this study and were procured from in-house animal house, Drugs Testing Laboratory Bangalore. They were fed with standard diet and water and housed in cages at room temperature (30±2°C) with a 12 h light and dark cycle. Ethical clearance required for the animal experiment was obtained from institute animal ethical committee (IAEC) (MIP/IAEC/2016-17/M1/07).

Excision Wound Model:
The excision wound model was carried out in eight groups of rats (n =6) and treatments were as follows: group 1 Control, group 2 was treated by standard, Groups 3,4 treated by 5% and 10% ointments, Groups 5,6 treated by 5% and 10% Creams, Groups 7,8 treated by 5% and 10% gel,. A circular wound of 2.5 cm diameter made on the depilated dorsal thoracic region of the rat under pentobarbitone anaesthesia (60 mg/kg, ip) under aseptic conditions. The area of the wound will be recorded on transparency paper. The animals of treatment group received the drug twice daily. On 4th, 8th, 12th, 21st day wound area were measured and recorded. On the 21st day the newly formed tissue was carefully excised from the rat back under anaesthesia. Wound biopsies were fixed in 10% formalin solution and sections (4 mm) were cut and stained with haematoxylin and eosin. Sections were histopathologically assessed under light microscope and graded in respect of re-epithelization, vascularity and fibroblast content. The wound area was observed.

In the other biopsy collagen content was estimated by Sircol reagent kit. Acid soluble collagen was estimated. Briefly, punched skin was mixed with 500 µl of 0.5M acetic acid and homogenized and centrifuged at 19,000 rpm for 30 min. To a 10 µl aliquot of supernatant, 90 µl of 0.5M acetic acid and 1 ml of Sircol reagent were added (Sircol collagen assay kit) and vortexed for 30 min. It was re-centrifuged at 19,000 rpm for 30 min. The supernatant was decanted and the pellet was reconstituted in 2 ml of 0.5M NaOH. The color complex was measured at 540 nm.

Statistical test:
Statistical differences between absolute data of control and treated groups were tested by one way ANOVA followed by Dunnett's test. The difference were very significant at ($p<0.01$) significant ($p<0.05$).

Results:
Wounds treated groups exhibited marked dryness and there was no visual sign of inflammation or any pathological fluid oozing out from the wounds as compared to control treated wounds.

The wound healing effect of METP at the strength of 5% formulations treated of rat was statistically significant ($P<0.05$) after 21 days but the strength of 10% formulations and silversulphadiazine treated of rats were very significant ($P<0.01$) in excision wound model dose dependently (Table-1). The percentage wound inhibition was close to standard Silver sulphadiazine (Table-2).

In excision wound model of rats the collagen content was estimated by using kit and the values are tabulated in (Table 4). Wounds treated METP (5%) formulations exhibited a statistically significant collagen levels as compared to control group ($P<0.05$). However, the collagen content of wounds treated with METP (10%) and Silver sulphadiazine were statistically very significant ($P<0.01$), as compared to control wounds dose dependently.

Histopathological relative grading of dermal sections findings revealed that as compared to control group, topical application of METP formulations (10%) on dermal wounds increased fibroblasts and re-epithelization with moderate vascularity (Table-3) as compared to control group dose dependently.

The Order of potency is Silver sulphadiazine> METP Ointment base > METP Cream base > METP Gel base.

Table 1: Effect of extracts of different formulations of METP on excisional wound model in rats:

SL.NO	Groups	Wound area (mm)			
		Day 4	Day 8	Day 12	Day 21
1	Control	24.23± 0.18	24.01± 0.12	23.92± 0.11	20.27± 0.13
2	Silver sulphadiazine (1%)	23.82± 0.12	20.23± 0.14	16.88± 0.17*	10.35± 0.12**
3	METP Ointment base (5%)	24.35± 0.17	23.18± 0.17	19.78± 0.16	16.96± 0.14*
4	METP Ointment base (10%)	23.11± 0.19	21.34± 0.16	17.18± 0.14*	13.01± 0.16**
5	METP Cream base (5%)	24.12± 0.13	22.93± 0.12	18.99± 0.16	17.29± 0.15*
6	METP Cream base (10%)	22.89± 0.21	21.87± 0.15	16.01± 0.15*	13.98± 0.11**
7	METP Gel base (5%)	22.78± 0.17	21.22± 0.14	18.91± 0.18	17.22± 0.16*
8	METP Gel base (10%)	22.97± 0.12	21.82± 0.19	15.09± 0.11*	14.01± 0.19**

All expressed as mean and standard error mean (S.E.M). Mean in columns with different letters were significant *P < 0.05 Very significant **P < 0.01)

Table 2: Percentage inhibiton of activity of METP

% Inhibition				
Days/Formulations	Day 4	Day 8	Day 12	Day 21
Silver sulphadiazine (1%)	1.69	15.74	29.43	48.94
METP Ointment base (5%)	-0.50	3.46	17.31	16.33
METP Ointment base (10%)	4.62	11.12	28.18	35.82
METP Cream base (5%)	0.45	4.50	20.61	14.70
METP Cream base (10%)	5.53	8.91	33.07	31.03
METP Gel base (5%)	5.98	11.62	20.94	15.05
METP Gel base (10%)	5.20	9.12	36.91	30.88

Table 3: Relative grading of Dermal sections on histology findings at 21 day post wounding in excision wound model in rats.

Group	Re-epithelization	Fibroblast number	Vascularity
Control	++	+	+
Silver sulphadiazine (1%)	+	+++	+++
METP Ointment base (5%)	++	++	+
METP Ointment base (10%)	+	+++	+++
METP Cream base (5%)	+	++	+++
METP Cream base (10%)	+	+++	+++
METP Gel base (5%)	+	++	+++
METP Gel base (10%)	+	+++	+++

+ Slight; ++ Moderate; +++ Marked

Table 4: Collagen content of dermal sections from rats of different groups at 21 days post wounding in excision wound model.

Groups	Collagen (µg)
Control	28.2±2.1
Silver sulphadiazine (1%)	49.24±2.1**
METP Ointment base (5%)	36.8±2.6 *
METP Ointment base (10%)	42.7±1.9**
METP Cream base (5%)	38.9±5.2*
METP Cream base (10%)	41.1±4.1**
METP Gel base (5%)	37.2±2.1*
METP Gel base (10%)	40.2±2.5**

All expressed as mean and standard error mean (S.E.M). Mean in columns with different letters were significant *P < 0.05 Very significant **P < 0.01

Discussion and Conclusion:
Wound healing is a complex multiphase process that involves a chain of well-orchestrated biochemical and cellular events. The process can be broadly classified in three stages- inflammation, proliferation and remodelling. The participation of various inflammatory cells is crucial for repair process. These cells promote migration and proliferation of endothelial cells, leading to neovascularization[1]. The proliferative phase is characterized by angiogenesis, collagen deposition, granulation tissue formation, epithelisation and wound contraction. Finally the fibroblasts grow and form extracellular matrix as part of tissue remodeling. These interlinked events are controlled by specific growth factors and cytokines at site of injury[14]. Impaired wound healing causes morbidity for patient and may lead to complication like-dehiscence and chronic wound healing ulcer[15]. Currently, the mainstay of treatment modality is steroid application with supportive antibiotics, which is fraught with unwanted side effects. Therefore, there is a need to develop therapeutic agents, which augment healing process. leaves of *Tridax procumbens* by way of pharmacological assays. In excision wound model, methanollic extracts of leaves of *Tridax procumbens* was administered intraperitoneally to rabbits. An

equivocal response was elicited wherein enhanced re-epithelization of wound was recorded on one hand, and retardation of scar contraction and granulation, on the other. It was postulated that extract of *Tridax procumbens* is essentially prohealing but also has corticotropic influence (as evidenced by increase in adrenal weight and decrease in thymus weight in rat experiments). The corticotropic effect might indirectly retard the healing process by enhancing endogenous secretion of cortical hormones, which are known to inhibit all the phases of wound healing. The dermal wound healing property of leaves of *Tridax procumbens* in the regional literature is well documented[4]. An equivocal response was elicited wherein enhanced re-epitheli zation of wound was recorded on one hand, and retardation of scar contraction and granulation, on the other. Topical formulations are preferred choice for healing dermal wounds as they are locally well absorbed to produce pharmacodynamic action effectively. Secondly, topical formulations help to circumvent adverse events associated with systemic administration of the drug and it was hypothesized that the reported antihealing effects of *Tridax procumbens* could be attenuated by this approach. In addition, this approach is in tandem with the traditional use of this medicinal plant involved topical application and is cited to be effective. We report here that as a topical formulation, METP (10%) was found to be effective in healing dermal wound was compared with control. METP (10%) acted by stimulating collagen synthesis, which has been reported to be an essential step in faster healing of wound. Further, histopathological evaluation of dermal wounds indicated fibroblast number proliferation accompanied with neovascularization in the METP (10%) treated group. Wound healing in any tissue follows a predictable sequence of events with the aim to restore damage tissue as closely as possible to its normal state. This study clearly demonstrates that METP augments proliferation and remodelling stages of wound healing. However, this is a dose dependent phenomenon, as the higher dose exhibited antihealing property, which is in confirmation with earlier reported observations.

Therefore, the present study was conducted to unequivocally explore the effect of *Tridax procumbens* (METP) on dermal wound healing. The study was designed to investigate the effectiveness of topical different formulations. The Order of potency is Silver sulphadiazine> METP Ointment base > METP Cream base > METP Gel base. Further studies are required to delineate the mechanism underlying the anti-healing effects of *Tridax procumbens*.

References:

1. Clark RAF. Cutaneous wound repair. New York: Oxford University;1991. p. 576.
2. Barua CC, Talukdar A, Begum SA, Sarma DK, Fathak DC, Barua AG, et al. Wound healing activity of methanolic extract of leaves of Alternanthera brasiliana Kuntz using in vivo and in vitro model. Indian J Exp Biol 2009;47:1001-5.
3. Christudas S, Kulathivel TM, Agastian P. Phytochemical and antibacterial studies of leaves of Tridax Procumbens L. Asian pacific journal of tropical medicine, 2012; S159-S161.
4. Ali M, Rawinder E and Ramachamdram R: A new flavonoid from the aerial parts of Tridax procumbens L. Fitoterapia 2001;72:313-315.
5. Durgacharan A Bhagwat, Suresh G Killedar, Rahul S Adnaik. Anti-diabetic activity of leaf extract of Tridax procumbens. Int J Green Pharm 2008;2(2):126-128.
6. Chitra Pai, Ujjwala Kulkarni, Manjusha Borde, Sowmya Murali, P.Mrudula and Yashwant Deshmukh. Antibacterial Activity of Tridax procumbens with Special Reference to Nosocomial Pathogens. British J Pharm Res 2011;1(4):164-173.
7. Rappiah-Opong, AK Nyarko, D Dodoo, FN Gyang, KA Karam and NK Ayisi. Antiplasmodial activity of Extracts of Tridax procumbens and Phyllanthus Amarus in in Vitro Plasmodium Falciparum Culture Syatems Ghana Med J 2011;45(4):143-150.
8. Reddipalli Hemalatha. Anti-hepatotoxic and anti-oxidant defense potential of Tridax procumbens. Int J Green Pharm 2008; 2(3):164-169.
9. Sneha Mundada and Ruchi Shivhare. Pharmacology of Tridax procumbens a Weed: Review Int J Pharm Tech Res 2010;2(2):1391-1394.
10. U Tiwari, et al. Immunomodulatory Effects of Aqueous Extract of Tridax Procumbens in Experimental Animals. J Ethnopharmacol 2004;92:113-119.
11. Vishnu Priya P, Radhika K, Sivakumar R, Sri Ramchandra M, Prameela Devi V, and Rao Srinivasa. Evaluation of AntiCancer Activity of T. Procumbens Flower Extracts on PC3 cell lines. International Journal of Advances in Pharmaceutical Sciences 2011;2(1):28-30.
12. Sahoo M and Chand PK. In vitro multiplication of a medicinal herb Tridax procumbens L. (Mexican Daisy, coat button): influence of explanting season, growth regulator synergy, culture passage and planting substrate. Phytomorphol 1998;48:195-206.
13. Pathak AK, Saraf S, Dixit VK. Analgesic activity of Calotropis gigantea flower. Fitoterapia 1991;62:307-13.
14. Bennet NT, Schultz GS. Growth factors and wound healing: Biochemical properties of growth factors and their receptor. Am J Surg 1993;165:728-737.
15. Goodson, Hunt TK. Wound healing and diabetic patient. Sur Gynecol Obst 1979;149:600-608.