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ACUTE DERMAL TOXICITY AND WOUND HEALING ACTIVITY OF *MIKANIA MICRANTHA* OINTMENT IN RATS

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ABSTRACT

BACKGROUND: The plant, *Mikania micranntha* Kunth. has been traditionally used as a wound healing drug since ancient times, but its scientific evaluation is not fully explored yet. The objective of our study was to evaluate acute dermal toxicity and wound healing activity of a *M. micrantha* ointment in rats.

METHODS: Excision wound model was used in current study. The effect of test drug was assessed from wound contraction.

RESULTS AND CONCLUSIONS: The *M. micrantha* ointment showed moderate rate of contraction of wound that was slightly less than that of the standard drug, mupirocin. The *M. micrantha* ointment was also non-toxic to rat skin, suggesting a good potential of developing an inexpensive *M. micrantha* ointment for promoting accelerated wound healing.

KEYWORDS

Ayurveda, the Indian traditional system of medicine mentions "Vrana" meaning discontinuation of membrane linings – wounds and considers wounds healing as an important process which play a vital role in survival [1]. Hence, it emphasizes the need of wounds healing that is restoration of damaged tissues in wound or fracture in clinical medicine. Although Ayurveda uses drugs originated from natural resources like plants, minerals, animals etc., phyto-medicines have immense potential for the management and treatment of wounds, because these are affordable and rarely induce hyper-sensitive reactions to non-targeted cells/organis [2,3]. Several plants are described to have such wound healing attributes in Ayurvedic armamentarium under the broad heading of Vrana Ropaka [4]. However, there is a need for scientific validation, standardization and safety evaluation of plants of the traditional medicine for recommending them as wounds healing formulations.

Mikania micrantha Kunth (family Asteraceae, local name in West Bengal: tarulata, banchchalata, germalata) is bestowed with several desirable health benefits. The Mikania genus is one of the best-selling medicinal climbers in the world [5]. The plant is credited with various medicinal properties and is widely used by local practitioners of several countries as phytotherapeutics [6,7]. The plant has been reported to possess antifungal, antibacterial, antimicrobial and protein phosphatase-1 (PP1) inhibitory activities [8-12]. Its leaves are used as poultice for wound healing. The hypoglycaemic, antioxidant and wound healing properties of its ethanolic leaf extract have also been established in rat models [13,14]. Our institute is actively engaged in developing herbal drugs against various diseases. In the present studies, we formulated an ointment, using *M. micrantha* whole plant juice and evaluated its wound healing potential as well as dermal toxicity. Herein, we report the results.

MATERIALS AND METHODS:

Chemicals Xylazine hydrochloride (Stanex Drugs Chem. Pvt. Ltd. Hyderabad, India), lignocaine hydrochloride injection I.P (Lox 2%, NEON Lab. Ltd., Mumbai, India), mupirocin I.P (2% w/w, WALLACE Pharm. Pvt. Ltd., Goa, India) and vaseline (Cool Cosmetic Pvt. Ltd., Tamil Nadu, India) were used for the studies.

Plant material

Fresh *M. micrantha* plants were collected from its natural habitat of West Bengal, India in the month of April, 2018. The plant was identified and authenticated by Pharmacognosist. The collected whole plant was washed and juice was extracted as described in the following.

Preparation of plant juice

The whole plant (2 kg) were mixed with water (200 ml) and grinded in a mechanical grinder to obtain the fresh juice (800 ml).

Preparation of the ointment

The above juice was dried in a hot chamber at 40 °C to obtain a concentrate (100 g). This was powdered and thoroughly mixed with vaseline (1:1 w/w) with the help of a morter and a pastle to obtain the ointment as a homogenous dispersion. This was stored in an airtight container.

Animals

Male wistar strain albino rats, weighing 200 ± 20 g were used as per the guidelines of the Institutional Animal Ethics Committee (IAEC). The animals were obtained from the animal house attached to the pharmacology laboratory, CARIDD, Kolkata. The animals were maintained under ideal husbandry conditions in terms of standard conditions of temperature (23 ± 2 °C), relative humidity (60 – 70%) and exposed to 12 h light and dark cycles. All animals were housed in polypropylene cages, exposed to the same environmental conditions and fed with standard diet (National Institute of Nutrition, Hyderabad) and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee (05/P/S/IAEC/2017, dated 27th April 2017.) as per guideline of Committee for the Purpose of Control and Supervision on Experiments on Animals, India.

Dermal toxicity study

The selected animals were acclimatized in laboratory conditions for seven days. Twenty four hours prior to the external application of test drug, fur at the back of the each animal was shaved off from about 10% of total body surface area and they were randomly divided into three

groups with six animals in each group. Studies were carried out with three different types of bandages, prepared using vaseline, mupirocin and *M. micrantha* ointment-coated gauges. The bandages were wrapped around the shaved off skins parts of the rats for 24 h, before removing the bandages. Group I animals, receiving vaseline served as the control. The groups II and III rats were applied mupirocin *and M. micrantha* ointment respectively. The animals were kept individually in separate cages and were observed daily for 2 weeks for any signs of toxicity and also changes in skin, mucous membrane, fur, behavioral pattern [15].

Excision wound model

The method described by Saha et al. (1997) was adopted for the study. Back of five rats in each group was depilated and the areas were cleaned with 70% alcohol under xylazine hydrochloride (10 mg/kg intraperitoneally) anaesthesia and local infiltration of lignocaine hydrochloride. Excision wound was inflicted by cutting away a 200 mm^2 full thickness of skin from a predetermined area; the wound was left open. The rats were randomly divided into three groups with six animals in each group as per the following.

Group I :

Treated with plain vaseline (ointment base) and served a control-I.

Group II :

Treated with mupirocin ointment.

Group III :

Treated with *M. micrantha* ointment formulation.

The test ointments and the blank ointment base for the purpose of control were topically applied for 7 days and thereafter the progressive changes in wound area were measured by manual vernier caliper every alternate day upto 21 days. This model was used to monitor wound contraction and closure time. Wound contraction was calculated as percent reduction in wound area [16].

RESULTS AND DISCUSSION

Acute dermal toxicity of the ointment

In order to develop an inexpensive, but effective wound healing drug from *M. micrantha*, the whole plant juice (Swaras) was mixed with vaselin to obtain an ointment. Evaluation of acute dermal toxicity is essential for developing any wound healing formulation [17,18]. Hence we first evaluated the above parameter of the newly developed *M. micrantha* ointment following OECD guidelines [16] and compared the results with that of vaseline (used as the base for the herbal ointment) and mupirocin ointment as the positive control. Rats of all the groups did not show any behavioural changes and other parameters during the entire experimental period of 14 days. Rats in all the groups were equally alert, painless to touch, able to feed themselves well. There were no irritation signs on the skin, erythema, eschar, edema, exudates, fluid secretion, or any other reactions in either intact or abraded sites of all rats. There were no abnormal findings from a gross pathological

Wound healing activity

Topical application of the herbal ointment at the wound sites in excision wound healing model produced significant ($p < 0.001$) wound healing. Due to natural healing, wound contraction was observed even in the control (vaseline) group, suggesting acute nature of the wounds. But the healing process in this animal group was very slow, and ~50% wound closure was observed on the 15^{th} day of wound induction. Treatment with mupirocin and *M. micrantha* ointment accelerated wound contraction, leading to faster healing compared to the control group. Moderately higher rate of contraction was found in the mupirocin group *vis-à-vis M. micrantha* group (Table 1). The healing results of the different groups are graphically shown in Figure 1. Wound healing activity of the plant juice was also confirmed by measuring the tensile strength. An increase in tensile strength of the treated wounds may be due to an increase in collagen concentration and stabilization of the fibers facilitating wound healing.

Wound healing is a complex process of restoring cellular structures and tissue layers in damaged tissues to their normal states. Wound contracture occurs throughout the healing process, commencing in the fibroblastic stage whereby the area of the wound undergoes shrinkage. It has 3 phases; inflammatory, proliferative and maturational, and is dependent upon the type and extent of damage, the general state of the host's health and the ability of the tissue to repair. The inflammatory phase is characterized by hemostasis and inflammation, followed by epithelization, angiogenesis, and collagen deposition in the proliferative phase. In the maturational phase, the final phase, the wound undergoes contraction resulting in a smaller amount of apparent scar tissue. Granulation tissues formed in the final part of the proliferative phase are primarily composed of fibroblasts, collagen, edema, and new small blood vessels. The increase in dry granulation tissue weight in the test treated animals suggestsed higher protein content [17]. The day-wise macroscopic images (Figure 2) of the wound areas in the three animals groups revealed significant wound healing by the *M. micrantha* ointment.

CONCLUSION

The present study demonstrated that an ointment of *M. micrantha* juice has accelerated wound healing property, which was almost comparable with the standard wound healing ointment, mupirocin. Although different *M. micrantha* extracts have earlier shown wound healing in rats [13,14], to the best of our knowledge, this is the first report of a *M. micrantha* juice-based ointment as an effective topical treatment for management of exclusion wounds.

Table 1: Wound healing effect of vaseline, mupirocin and *M. micrantha* ointment on excision wound (Sq.cm) model in Rats.

GROUP	3rd day (Mean ±SD)	6th day (Mean ±SD)	9th day (Mean ±SD)	12th day (Mean ±SD)	15th day (Mean ±SD)	18th day (Mean ±SD)	21st day (Mean ±SD)	p-value
Group-I (Vaseline)	1.83±0.06	1.67±0.07	1.43±0.06	1.23±0.06	0.9±0.1	0.73±0.1	0.53±0.6	<0.01
Group-II (Mupirocin)	1.60±0.10	1.3±0.10	1.03±0.19	0.67±0.06	0.47±0.08	0.25±0.05	0.03±0.6	<0.001
Group-III (Mikania micrantha)	1.67±0.15	1.49±0.13	1.23±0.20	0.8±0.18	0.6±0.13	0.35±0.05	0.21±0.3	<0.001
p-value	0.09	0.01	0.046	0.0022	0.0062	0.0004	P<0.0001	

Figure I: Effect of vaseline, mupirocin and M. micrantha ointment on Excision wound (Sq.cm) model in Rats

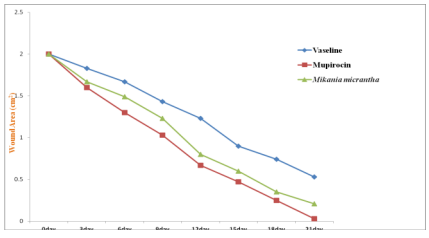
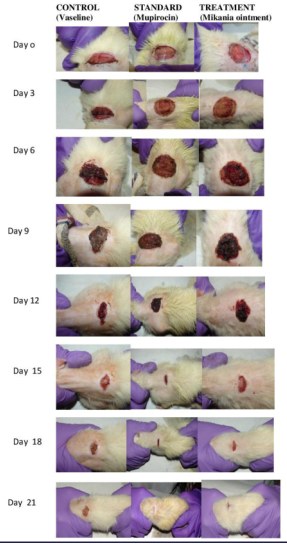


Figure 2: Macroscopic view of rat wounds



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