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KEY WORDS: Areca catechu, Wound area, Fibroblast number and Collagen content.

Evaluation of Wound Healing Potential of Different Topical Formulations of Methanolic Nut extracts of *Areca Catechu* Linn. in Rats

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ABSTRACT

Aim: Areca nut (Areca catechu Linn.) is one of the commonly used Indian traditional medicines for skin ulcers. The present study was carried out to evaluate the effect of Areca catechu extracts of topical formulations on wound model in rats. Methods: The present study evaluates the effect of topical treatment in different formulations like Ointments (5% & 10%), Creams (5% & 10%) and Gels (5% &10%) of the Methanolic nut extracts of Areca catechu (MEAC) using excision wound model in rats. Excision wounds (2.5 mm, i.d.) were inflicted on depilated back of rats. Formulations of MEAC were applied twice daily for 21 days on the dermal wound. The parameters observed were Wound area, re-epithelization, vascularity, fibroblast number and collagen content. Results & Conclusion: The MEAC of Ointment (10%), Creams (10%), Gels (10%) and Silver sulphadiazine (1%) showed statistically very significant (P>0.01) when compared to control group at 21st day. The overall results imply that MEAC possesses dose dependent pro-healing potential. The Order of potency is Silver sulphadiazine> MEAC Gel base > MEAC Cream base > MEAC Ointment base.

Introduction:

Wounds are physical injuries that result in an opening or break of the skin. Proper healing of wounds is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin[1]. Wound may be produced by physical, chemical, thermal, microbial or immunological insult to the tissue [2]. Wound healing is the process of repair that follows injury to the skin and other soft tissues. Wound healing involves continuous cell–cell and cell–matrix interactions that allow the process to proceed in three overlapping phases viz. inflammation (0–3days), cellular proliferation (3–12 days) and remodeling (3–6 months). Cutaneous wound are characterized by migration and proliferation of fibroblasts, endothelial and epithelial cells, deposition of connective tissue, angiogenesis, re-epithelization, and finally contraction of wound[3]. Healing of wounds is one of the important areas of clinical medicines explained in many Ayurvedic texts under the heading "Vranaropaka". According to the Ayurveda, Vrana (wounds or ulcers) is the discontinuation of lining membrane that after healing leaves a scar for life closely resembling the modern definition[4]. Similarly, inflammation is considered to be an early phase in the pathogenesis of wounds termed Vranashotha. The objective in wound management is to heal the wound in the shortest time possible, with minimal pain, discomfort, and scarring to the patient[4,5]. Plants and their extracts have immense potential for the management and treatment of wounds. The phyto-medicines for wound healing are not only cheap and affordable but are also safe as hyper sensitive reactions are rarely encountered with the use of these agents [2]. These natural agents induce healing and regeneration of the lost tissue by multiple mechanisms. However, there is a need for scientific evaluation of plants of the traditional medicine before these could be recommended for healing of the wounds. *Areca catechu* is commonly called betel nut, belongs to the family Aereaceae. *Areca catechu* is used as hepatoprotective, anthelmintic, antidiabetic, antioxidant, antibacterial, antiulcerogenic, antifertility, abortifacient, antiviral, anti- implantation, antivenom, anti-inflammatory and anticonvulsant drugs [6,12]. The seed contains 50-60% sugars, 15% lipids 15% condensed tannins, polyphenolics and 0.2 - 0.55% alkaloids[13]. Phytochemical analysis of *Areca catechu* revealed that the presence of arecaidine, arecaine, arecoline, b-carotene, b-sitosterol, capric acid, D-catechin, gallic acid, guvacine, guvacoline, heneicosanic acid, isoguvacine, kryptogenin, lauric acid, leucocyanidine, leucopelargonidine, linoleic acid, margarinic acid, myristic acid, oleic acid, philobaphenetannin and stearic acid[14]. The anthelmintic activity of Areca catechu aganist Haemonchous contortus has been reported [15]. The present study focussed on wound healing potential of different topical formulations of methanolic nut extracts of *Areca Catechu* Linn. in rats

Methodology:

Plant material, extract and formulation preparation:

Nuts of Areca catachu L. were collected from different localities of Bangalore and its nearby areas and washed thoroughly with distilled water. The cleaned nuts 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:
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 was 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 MEAC 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 MEAC (5%) formulations exhibited a statistically significant collagen levels as compared to control group ($P<0.05$). However, the collagen content of wounds treated with MEAC (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 MEAC 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> MEAC Gel base > MEAC Cream base > MEAC Ointment base.

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

SL.NO	Garoups	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	MEAC Ointment base (5%)	24.84± 0.15	23.01± 0.21	19.82± 0.15	16.85± 0.23*
4	MEAC Ointment base (10%)	23.72± 0.15	20.54± 0.22	15.65± 0.14*	12.05± 0.16**
5	MEAC Cream base (5%)	23.21± 0.22	21.52± 0.18	18.32± 0.17	16.92± 0.26*
6	MEAC Cream base (10%)	22.99± 0.14	22.21± 0.16	16.32± 0.13*	12.98± 0.15**
7	MEAC Gel base (5%)	24.25± 0.19	22.97± 0.18	20.32± 0.18	17.03± 0.16*
8	MEAC Gel base (10%)	23.31± 0.22	21.85± 0.19	17.15± 0.18*	13.01± 0.17**

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 MEAC

% Inhibition				
Days/Formulations	Day 4	Day 8	Day 12	Day 21
Silver sulphadiazine (1%)	1.69	15.74	29.43	48.94
MEAC Ointment base (5%)	0.43	4.29	11.46	18.61
MEAC Ointment base (10%)	5.37	8.59	26.56	38.28
MEAC Cream base (5%)	6.01	11.78	14.79	20.29
MEAC Cream base (10%)	5.55	9.05	28.81	33.71
MEAC Gel base (5%)	-0.5	3.43	16.33	17.31
MEAC Gel base (10%)	4.66	11.39	28.13	35.82

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%)	+	+++	+++
MEAC Ointment base (5%)	++	++	+
MEAC Ointment base (10%)	+	+++	+++
MEAC Cream base (5%)	+	++	+++
MEAC Cream base (10%)	+	+++	+++
MEAC Gel base (5%)	+	++	+++
MEAC 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	38.2±2.1
Silver sulphadiazine (1%)	49.24±2.1**
MEAC Ointment base (5%)	40.21±2.6 *
MEAC Ointment base (10%)	44.01±1.9**
MEAC Cream base (5%)	41.28±5.2*
MEAC Cream base (10%)	45.52±4.1**
MEAC Gel base (5%)	42.58±2.1*
MEAC Gel base (10%)	47.23±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 significan **P < 0.01

Discussion:
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 neovascularization3. 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 injury16. Impaired wound healing causes morbidity for patient and may lead to complication like-dehiscence and chronic wound healing ulcer[17]. 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.

The dermal wound healing property of nuts of Areca catechu in the regional literature is well documented9. 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. 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 Areca catechu could be attenuated by this approach9. 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, MEAC (10%) was found to be effective in healing dermal wound was compared with control. MEAC (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 MEAC (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 MEAC 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. Further studies are required to delineate the mechanism underlying the anti-healing effects of Areca catechu

Conclusion:

MEAC possesses dose dependent pro-healing potential. The Order of potency is Silver sulphadiazine> MEAC Gel base > MEAC Cream base > MEAC Ointment base. The study was designed to investigate the effectiveness of topical Different formulations.

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