Research Article

Areca catechu: Effect of topical ethanolic extract on burn wound healing in albino rats

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ABSTRACT

Background: Areca nut (*Areca catechu Linn.*) is commonly used for skin ulcers in Indian traditional medicine. Areca nut oil is prepared and is applied topically for burn wound healing. However, scientific study has not been conducted so far. Hence, this study was aimed to evaluate burn wound healing activity of *Areca catechu*.

Objective: To evaluate the burn wound healing property of *Areca catechu* kernel in normal as well as dexamethasone treated rats.

Materials and Methods: Ethanolic extract of *Areca catechu* kernel was prepared and an ointment was made with 2% of this extract. Burn wound was induced by standard procedure. Rats with burn wound model received either vehicle, standard drug or test drug. Two other groups were injected with dexamethasone to delay the wound healing process. The dexamethasone treated groups received either vehicle or test drug topically. Wound contraction rate and period of epithelialization were measured. The collected data were subjected to statistical analysis.

Results: The wound contraction rate was significantly increased in *Areca catechu* treated group in all the days compared to control. Period of epithelialization was faster in the drug treated group than control group. The dexamethasone treated group showed a significant delay in wound healing process when compared to control. Test drug showed a significant reversal in wound contraction rate and epithelialization period in dexamethasone suppressed burn wound healing model.

Conclusion: This study has shown the wound healing property of *Areca catechu.* Further study is required to know the compounds responsible for its wound healing property and to understand the mechanism of action.

Key words: *Areca catechu*, burn wound, epithelialization, wound contraction, Ayurveda, Siddha

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INTRODUCTION

Areca nut (*Areca catechu Linn.)* is most commonly chewed as a constituent of betel quid which is a mixture of betel leaf, areca nut and slaked lime. Traditional medicines like Ayurveda and Siddha use areca nut as one of the ingredient in medicated oils used for the treatment of burn wound. It is a powerful sialagogue, and stimulates the secretion of sweat in the same way as pilocarpine. It contains catechin, tannins, gallic acid, fat, gum, alkaloids like arecoline and arecaine. Arecaidine, guvacoline, guvacine and choline are present in trace amount.[1] Its antibacterial, antioxidant, wound healing,[2] hepatoprotective,[3] hypoglycemic,[4] antiulcerogenic,[5] antifertility,[6] abortifacient and anti-implantation[7] activities were already reported in animal studies. Since, no reports are available regarding the burn wound healing effect of alcoholic extract of *Areca catechu*, we undertook this study.

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MATERIALS AND METHODS

Experimental animals

Experimental protocol was approved by Institutional animal ethics committee (IAEC/ KMC/45/2011-2012), Manipal. Healthy, inbred, male albino Wistar rats weighing between 250-300 g were used. The rats were maintained under standard conditions in animal house approved by the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA). Rats were housed under controlled conditions of temperature 23 ± 2°C, humidity 50 ± 5% and 12 h light and dark cycles respectively. Animals were housed individually in polypropylene cages containing sterile paddy husk as bedding after making burn wound till completion of wound healing. Animals were maintained on standard rat feed (Amrut lab animal feed, India) and water *ad libitum*.

Drugs and chemicals

Ketamine injection was obtained from Neom laboratories Limited (Mumbai, India). Silver sulfadiazine (0.5 g of 1% cream) was obtained from Kasturba Hospital Pharmacy (Manipal, India). Dexamethasone was obtained from Nice Chemials Ltd (Cochin, India). The kernel of *Areca catechu* were purchased from local market and authenticated by the Professor of Botany, Mahatma Gandhi Memorial College, Udupi. Voucher specimen was kept in the Department of Pharmacology, KMC, Manipal. Ointment of ethanol extract (2%) was prepared from *Areca catechu*.

Preparation of ethanolic extract of Areca catechu kernel

The kernel of *Areca catechu* were chopped into small pieces and dried under sun for a few days. The dried chopped kernel was powdered and defatted in petroleum ether. The residue was hot extracted in soxhlet apparatus (Tensil Glass Works, Bangalore, India) using 400 mL of 70% ethanol for 5 cycles. The extract was filtered, lyophilized and concentrated over a water bath to obtain dry extract.[5] This crude extract was stored in a desiccator. For topical application, ointment of ethanolic extract (2%) was prepared using simple ointment base.

Study design

Burn wound healing property was studied in two different models. In both models, the drug treatment was started on day one and continued till falling of eschar leaving no raw wound behind. Three groups of six animals in each were used to evaluate burn wound healing property of *Areca catechu* extract. Control group, test group and standard group received topically, ointment base, 2% *Areca catechu* extract and silver sulfadiazine cream respectively.

Burn wound model

Partial thickness burn wounds were made on overnight fasted rats under ketamine (50 mg/kg, i.m.) anesthesia by pouring hot molten wax (2 g) at 80°C. The wax was poured on the shaven back of the animal through a cylinder of 300 mm^2 circular opening. The wax was allowed to remain on the skin for 8 minutes by that time it got solidified. This was considered as day 0.[8]

Dexamethasone suppressed burn wound model

Partial thickness burn wounds were made as mentioned above. In addition, dexamethasone was administered from day 0 (0.17 mg/kg, i.p.) and was continued on subsequent days till the day of eschar falling.[9]

In dexamethasone suppressed burn wound model, two groups were used. Control group received ointment base and test group received 2% *Areca catechu* extract, both were used topically.

Evaluation of burn wound healing activity

Wound contraction rate and epithelialization period were the two parameters assessed to evaluate burn wound healing activity of *Areca catechu* extract.

Wound contraction rate

Wound area was measured by tracing the wound size on a transparent butter paper on every alternate day of post wounding. The tracing

was then transferred to 1mm^2 graph sheets, from which the wound area was calculated. The calculated surface was then employed to calculate the percentage of wound contraction. By taking the initial size of wound 300 mm^2 as 100%, wound contraction rate was calculated by using the following formula;

$$\text{Wound contraction (\%)} = \frac{(\text{Initial wound size} - \text{specific day wound size})}{\text{Initial wound size}} \times 100$$

Period of epithelialization

Falling of the eschar after leaving no raw wound behind was taken as end point of complete epithelialization and the days required for this was taken as period of epithelialization.

Statistical Analysis

Results were expressed as mean ± SEM. The differences between experimental groups were compared using one-way Analysis of Variance (ANOVA) followed by Tukey's post-hoc test. The level of significance was set at $p < 0.05$.

RESULTS

Burn wound model

The wound contraction rate was significantly hastened in *Areca catechu* treated group in all the days compared to control, viz. day 5 ($p = 0.045$), day 7 ($p = 0.031$), day 9 ($p = 0.024$) and day 11, 13 and 15 ($p < 0.0001$). The period of burn wound epithelialization ($p < 0.0001$) was faster in the drug treated group than control group (Table 1, Figures 1 - 2).

Dexamethasone suppressed burn wound model

Administration of dexamethasone has delayed the wound healing process. But, there was a significant increase in wound contraction rate in the drug treated group in all days ($p < 0.0001$) and the epithelialization period was faster ($p < 0.0001$) compared to control (Table 2, Figures 3 - 4).

Table 1: Effect of topical *Areca catechu* on wound contraction rate and epithelialization period of burn wound model in rats

Group (n=6)	wound contraction (%)							Epithelialization period (day)
	Day3	Day5	Day7	Day9	Day11	Day13	Day15	
Control	4.7 ± 0.25	9.41 ± 5.59	18.55 ± 0.86	25.75 ± 0.82	32.14 ± 0.87	45.37 ± 0.99	58.55 ± 1.18	24.33 ± 0.67
Standard	14.25 ± 5.02	23.91 ± 5.70‡‡	29.76 ± 5.82	37.56 ± 5.96	61.62 ± 5.55¶	81.10 ± 5.28¶	94.11 ± 3.10¶	15.67 ± 0.67¶
2% extract	17.39 ± 4.67	25.63 ± 3.43*	35.62 ± 4.37‡	45.12 ± 5.57**	67.90 ± 4.42¶	87.23 ± 3.83¶	94.76 ± 2.52¶	16.0 ± 0.45¶

Values are expressed in mean ± SEM
$^{\P}p < 0.0001$ vs. control
$^{**}p = 0.024$ vs. control
$^{*}p = 0.045$ vs. control
$^{\ddagger}p = 0.031$ vs. control
$^{\ddagger\ddagger}p = 0.028$ vs. control

Table 2: Effect of topical *Areca catechu* on wound contraction rate and epithelialization period in dexamethasone treated burn wound model

Group (n=6)	wound contraction (%)										Epithelialization period (day)
	Day3	Day5	Day7	Day9	Day11	Day13	Day15	Day17	Day19	Day21	
Dexa control	3.2 ± 0.17	6.41 ± 0.40	12.61 ± 0.59	19.82 ± 0.63	24.74 ± 0.67	37.82 ± 0.86	50.47 ± 1.29	66.39 ± 1.13	79.24 ± 1.30	84.82 ± 1.02	28.33 ± 0.42
Dexa + 2% extract	13.40 ± 3.70*	20.17 ± 2.73¶	28.20 ± 2.21¶	35.82 ± 2.20¶	49.87 ± 2.83¶	62.61 ± 2.12¶	69.00 ± 1.69¶	84.04 ± 2.10¶	98.26 ± 1.74¶	100± 0.00¶	19.33 ± 0.33¶

Values are expressed in mean ± SEM
Dexa – dexamethasone
$^{\P}p < 0.0001$ vs. Dexa control
$^{*}p = 0.01$ vs. Dexa control

Figure 1: Effect of topical *Areca catechu* on wound contraction rate in burn wound model

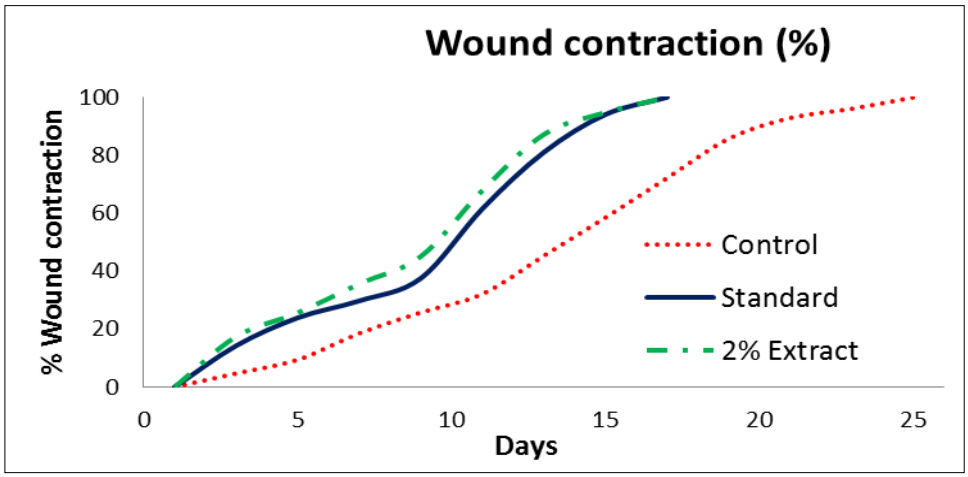


Figure 3: Effect of topical *Areca catechu* on wound contraction rate in dexamethasone treated burn wound model

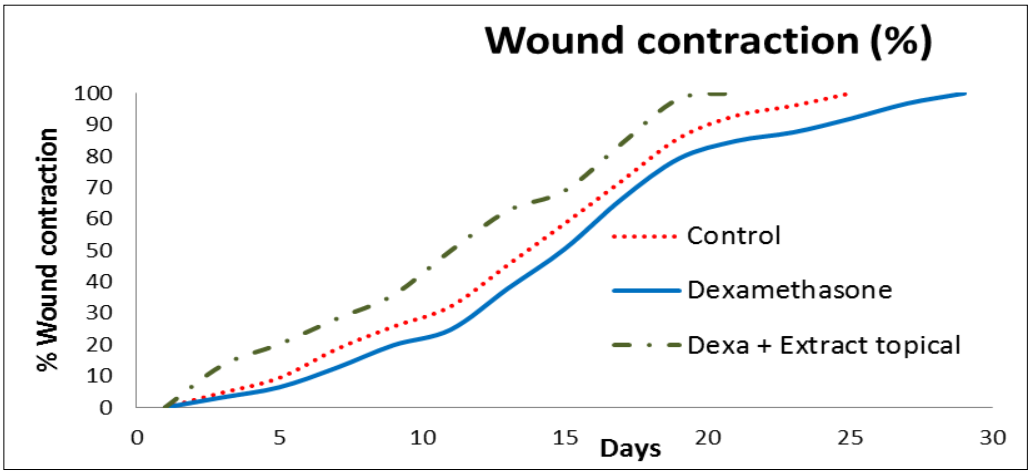


Figure 2: Effect of topical *Areca catechu* on epithelialization period in burn wound model

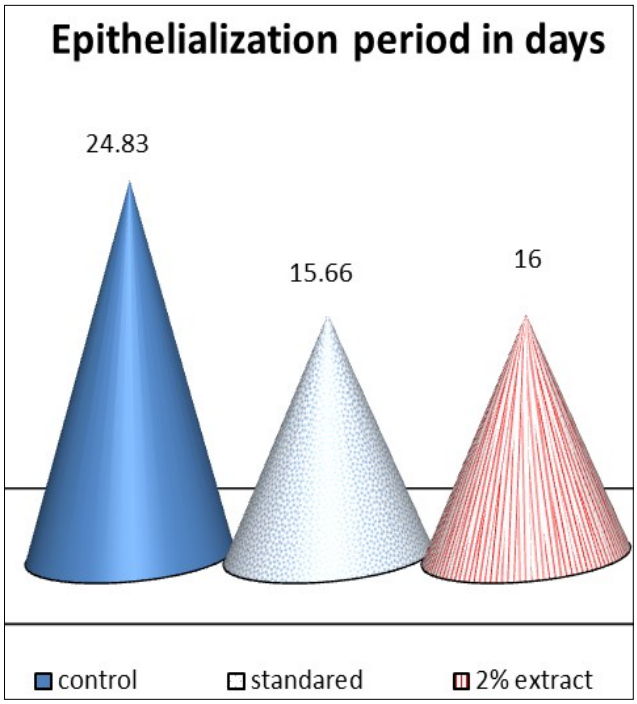
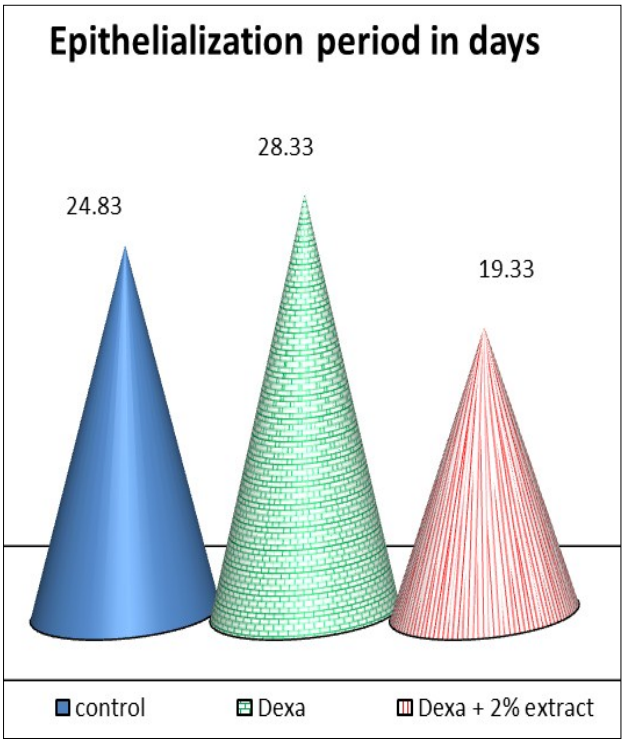


Figure 4: Effect of topical *Areca catechu* on epithelialization period in dexamethasone treated burn wound model



DISCUSSION

Burn wounds are commonly due to spillage of hot liquids. Most of the agents available are topical medicaments mainly aimed at preventing infection. Even though there are many wound healing enhancers, the results are not very satisfactory. Hence, there is always a need for better agent which can enhance healing as well as control infection.

Treatment of burn wounds has always been one of the most challenging clinical problems. Wound healing is a complex process, which helps to restore the damaged tissue as closely as possible to its original state.

Earlier report states that the polyphenols and alkaloid fractions of *Areca catechu* enhanced the healing of incision and excision wounds by increasing the breaking strength of granulation tissue.[2] In our study, there was a significant increase in the rate of burn wound contraction and period of epithelialization. Deaxmethasone inhibits wound contraction, granulation tissue and collagen formation. *Areca catechu* completely reversed the wound healing suppressive effect of dexamethesone.

It can be concluded that *Areca catechu* kernel favored burn wound healing and was also able to overcome the wound healing suppressive property of dexamethasone. It would thus be worth while to confirm the prohealing property of *Areca catechu* in diabetics and in patients on long term steroid therapy.

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