Research Article

Ethanolic Extract of Oral *Areca catechu* Promotes Burn Wound Healing in Rats

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ABSTRACT

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 oral *Areca catechu* extract on burn wound model in rats. To evaluate the burn wound healing activity of orally administered ethanolic extract of *Areca catechu* kernel in normal as well as dexamethasone treated rats. An oral formulation of ethanolic extract of *Areca catechu* was prepared using gum acacia. Rats with burn wound model received either vehicle (gum acacia, oral), standard drug (silver sulfadiazine, topical) or test drug (100 mg and 300 mg/kg extract, oral). Three other groups were treated with dexamethasone intramuscularly to delay wound healing process. The dexamethasone treated groups received either vehicle (gum acacia, oral) or test drug (100mg and 300mg/kg, oral). Parameters observed were wound contraction rate and period of epithelialization. *Areca catechu* has shown significant enhancement of wound contraction rate in all the days compared to control. Period of epithelialization was hastened in the drug treated groups. Dexamethasone suppressed wound contraction rate and epithelialization period were reversed significantly by oral *Areca catechu.* In this study, *Areca catechu* has shown the burn wound healing activity as well as reversed the dexamethasone suppressed wound healing.

Keywords: *Areca catechu*, burn wound, dexamethasone, epithelialization period, wound contraction.

INTRODUCTION

Areca nut (*Areca catechu Linn.)* chewing is a common practice in many parts of Asia, mainly India, Pakistan, Bangladesh and Sri Lanka. It is commonly chewed with betel leaf and slaked lime. Some of the traditional medicines use areca nut as one of the ingredient in medicated oils used for the treatment of burn wound. It is a powerful sialagogue. It contains catechin, tannins, gallic acid, fat, gum, alkaloids like arecoline and arecaine. Arecaidine, guvacoline, guvacine and choline are present in trace amount.[1] It has antibacterial, antioxidant, wound healing,[2,3] hepatoprotective,[4] hypoglycemic,[5] antiulcerogenic,[6] antifertility,[7] abortifacient and anti-implantation[8] activities. Topical *Areca catechu* extract has shown burn wound healing,[2] activity in rats. Hence, the present study was undertaken to assess the burn wound healing activity of areca nut extract by oral route in rats.

MATERIALS AND METHODS

Experimental animals

The study was undertaken after approval of experimental protocol by Institutional animal ethics committee (IAEC/KMC/45/2011-2012), Manipal. Healthy, male albino Wistar rats (250-300 g), bred locally in the animal house of Kasturba Medical College, Manipal were used. They were housed under controlled conditions of temperature 23 ± 2^{o} C, humidity 50 ± 5% and 10-14 h light and dark cycles respectively. Rats were housed individually in polypropylene cages containing sterile paddy husk (produced locally) as bedding throughout the experiment. They had free access to standard rat feed (Amrut lab animal feed, Pranav Agro Industries Ltd, Sangli, Maharashtra) and water *ad libitum* throughout the study.

Drugs and Chemicals

Ketamine injection (Neom Laboratories Ltd. Mumbai, India), Dexamethasone (Nice Chemicals, Cochin, India), gum acasia (Nice Chemicals, Cochin, India), Silver sulfadiazine (0.5 g of 1% cream) was obtained from Kasturba Hospital Pharmacy (Manipal, India). The areca nuts were purchased from a local shop and verification was done by the Professor of Botany, Mahatma Gandhi Memorial College, Udupi. Voucher specimen was kept in the department of Pharmacology, KMC, Manipal.

Preparation of ethanolic extract of *Areca catechu* kernel

The nuts of *Areca catechu* were cut into small pieces and dried for a few days. The dried pieces were 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.[7] This crude extract was stored in a desiccator. The two concentrations (100 and 300 mg/kg) of *Areca catechu* ethanolic extract were prepared using gum acacia.

Study design

Burn wound healing activity was studied in two different models. In both models, the drug treatment was started on day one and continued till the wound completely heal by falling eschar.

In *first model*, four groups of six animals in each were treated with 2 mL of 2% gum acacia (oral), 1% silver sulfadiazine cream (topical), 100 mg/kg and 300 mg/kg of ethanolic extract of *Areca catechu* (oral) respectively.

Burn wound model

Partial thickness burn wound was made on overnight fasted animals 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.[9]

Dexamethasone suppressed (second model) burn wound model

Partial thickness burn wound were made as mentioned above. In addition, dexamethasone was administered from day 0 (0.17mg/kg, i.p.) and was continued on subsequent days till the day of eschar falling.[10]

In dexamethasone suppressed burn wound model, *three groups* were used. They received oral gum acacia (control), other two groups received oral 100 mg and 300 mg/kg *Areca catechu* extract respectively.

Evaluation of burn wound healing activity

The two parameters assessed to check the burn wound healing activity were wound contraction rate and epithelialization period.

Wound contraction rate

Wound contraction rate was measured by planimetric measurement of wound area on alternate days of post wounding. This was done by tracing the wound on a transparent butter paper and then transferred to $1mm^2$ graph sheets. Reduction in the wound area was expressed as percentage of the original wound size.[11]

Period of epithelialization

The time taken for complete epithelialization was noted as number of days after wounding required for the eschar to fall off without any raw wound behind.

Statistical Analysis

All values were expressed as mean ± SEM. Data was analyzed using one-way Analysis of Variance (ANOVA) followed by Tukey's post hoc test. $P < 0.05$ was considered statistically significant.

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

Group (n=6)	Percentage of wound contraction (Mean ± SEM)								Period of epithelialization (days) Mean ± SEM
	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13	Day 15	Day 17	
Control	5.74 ± 0.34	9.2 ± 0.58	15.35 ± 0.55	27.04 ± 0.49	39.20 ± 0.56	52.20± 0.82	65.35 ± 0.99	74.61 ± 1.26	23.67 ± 0.42
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	100 ± 0.00 ¶	15.67 ± 0.67 ¶
100mg/kg extract	9.16 ± 1.50	17.88 ± 2.46	26.26 ± 2.92	35.36 ± 2.96	52.34 ± 2.96 †	72.34 ± 2.76 ‡‡	93.14 ± 2.22 ¶	100 ± 0.00 ¶	16.33 ± 0.42 ¶
300mg/kg extract	15.87 ± 333	25.99 ± 2.08 ‡	37.76 ± 1.27 ‡‡	47.08 ± 1.20 ¶¶	62.19 ± 1.96 ¶	77.08 ± 1.20 ¶	92.85 ± 1.20 ¶	98.24 ± 1.12 ¶	17.67 ± 041 ¶

¶ $P < 0.0001$ vs. control; ¶ ¶ $P = 0.002$ vs. control; ‡ $P = 0.009$ vs. control; ‡‡ $P = 0.01$ vs. control; * $P = 0.023$ vs. control; ** $P = 0.029$ vs. control; † $P = 0.049$ vs. control

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

Groups	Percentage of wound contraction (Mean ± SEM)										Epithelialization period (days) (Mean ± SEM)
	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13	Day 15	Day 17	Day 19	Day 21	
Dexa	3.20 ± 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 ± 042
dexa + 100mg extract	6.87 ± 0.58	14.07 ± 0.87 **	22.76 ± 1.16 *	27.71 ± 1.13 *	34.15 ± 0.99 *	51.39 ± 1.42 *	67.66 ± 2.38 *	82.93 ± 2.26 *	94.27 ± 1.91 *	100 ± 0.00 *	20.33± 0.42 *
Dexa + 300mg extract	14.00 ± 1.73 *,¶¶	23.36 ± 1.65 *, ¶	31.76 ± 1.10 *,¶	35.88 ± 0.63 *,¶	42.59 ± 1.22 *,¶	54.39 ± 0.97 *	69.94 ± 1.90 *	83.70 ± 2.57 *	93.17 ± 1.70 *	99.29 ± 0.71 *	21.33 ± 0.33 *

* $P < 0.0001$ vs. Dexa control; ** $P = 0.001$ vs. Dexa control; ¶ $P < 0.0001$ vs. Dexa + 100mg/kg; ¶¶ $P = 0.001$ vs. Dexa + 100mg/kg

RESULTS

Burn wound model

The wound contraction rate was significantly increased in *Areca catechu* treated groups compared to control throughout the study from day 5 onwards. The period of epithelialization was faster ($P < 0.0001$) in the drug treated groups (16 days in 100mg/kg and 17 days in 300mg/kg dose) than control group, which was 23 days (Table 1, Figure 1 and 2).

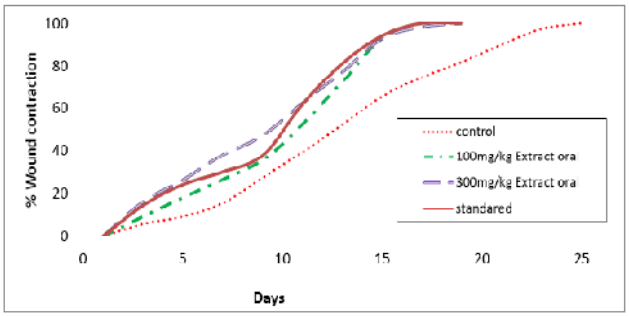

Figure 1: Effect of oral *Areca catechu* on wound contraction rate in burn wound model

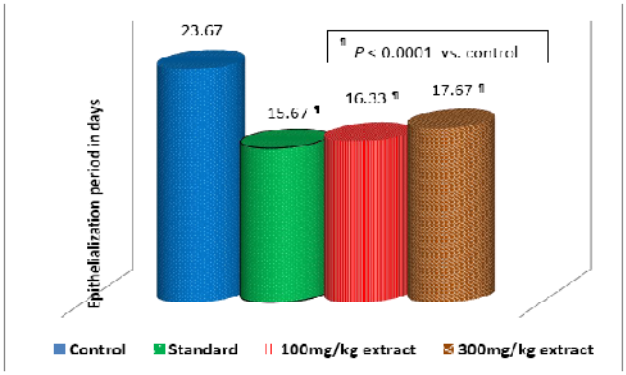

Figure 2: Effect of oral *Areca catechu* on epithelialization period in burn wound model

Dexamethasone suppressed burn wound model

In dexamethasone delayed burn wound model, wound contraction rate was significantly increased ($P < 0.0001$) by oral administration of *Areca catechu* in both the groups in all days. The mean period of epithelialization was significantly reduced ($P < 0.0001$) compared to Control (Table 2, Figure 3 and 4).

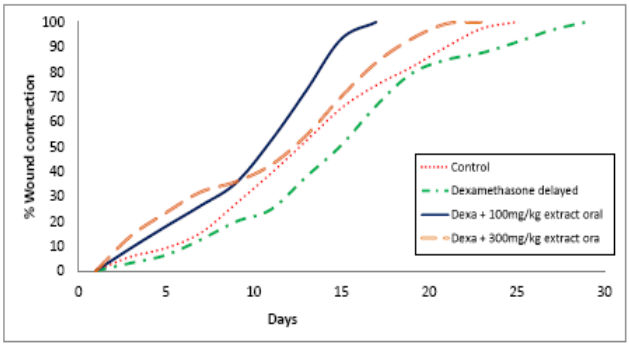

Figure 3: Effect of oral *Areca catechu* on wound contraction rate in dexamethasone delayed burn wound model

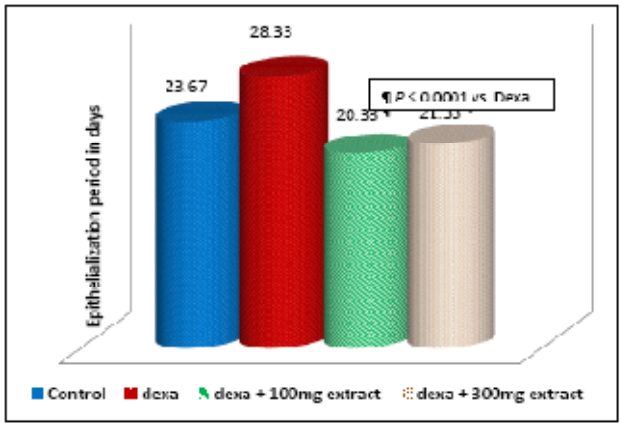

Figure 4: Effect of oral *Areca catechu* on epithelialization period in dexamethasone delayed burn wound model

DISCUSSION

Burn wounds are quite common which may be due to hot liquids, steam, fire, chemicals, electricity, etc. Most of the medicaments available are mainly aimed at preventing infection. Hence there is always a need for better agent, which can enhance healing as well as control infection.

The present study was undertaken to evaluate the effect of orally administered *Areca catechu* kernal on burn wound healing in normal as well as steroid treated rats. The ethanolic extract of orally administered *Areca catechu* hastened the burn wound healing process as well as completely reversed the wound healing suppressive effect of dexamethasone.

An earlier study report states, *Areca catechu* has polyphenols and some alkaloids which enhanced the healing of incision and excision wounds by increasing the breaking strength of granulation tissue.[3]

In our previous study, topical *Areca catechu* showed a significant increase in the rate of burn wound contraction and period of epithelialization in rats.[2] The present study (oral *Areca catechu*) demonstrated a significant improvement in the rate of wound contraction and period of epithelialization as well as reversed the wound healing suppressive effect of dexamethasone. This property could possibly be made use of clinically in patients as a supportive therapy to treat chronic wounds especially for diabetics. However, further studies are needed to support this suggestion.

CONCLUSION

In conclusion, this study showed oral *Areca catechu* extract promoted healing of burn wound in normal as well as in steroid suppressed rats.

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Dr. Arul Amuthan is an ethnopharmacologist, graduated in Siddha Medicine (Indian Traditional Medicine) from The Tamilnadu Dr. MGR Medical university. He pursued two post-graduation each in Siddha Medicine as well as Medical Pharmacology. He was awarded by Jawaharlal Nehru Memorial Fund for securing university first in his post-graduation at Manipal University. He is doing intense research in traditional medical systems in the area of drug standardization, drug discovery, pharmacological and toxicologicalevaluation.