

**EFFECT OF *PUNICA GRANATUM* PEEL EXTRACT ON BURN WOUND HEALING IN ALBINO WISTAR RATS**

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**ABSTRACT: Introduction:** *Punica granatum* is known to have long history of medicinal use. Aqueous fractions prepared from pomegranate peel extract has been found to stimulate type 1 procollagen synthesis and inhibit matrix metalloproteinase (which breaks down skin protein) produced by dermal fibroblasts.

**Objectives:** To study the effect of a topical *Punica granatum* peel extract on healing of burn wound and to compare its effect with that of standard (silver sulfadiazine).

**Materials and Methods:** Partial thickness burn wounds were inflicted upon wistar rats by pouring hot molten wax at 80°C in to a metal cylinder with 300mm square circular opening, placed on the back of the animal. There were four groups in the study viz. control (topical petroleum jelly), standard (topical Silver Sulphadiazine Cream) and test groups (topical *Punica granatum* peel extract 10% and 20 %). The parameters measured were wound contraction and epithelization period in this burn wound model.

**Results & Discussion:** The percentage of wound contraction was significantly increased in the topical *Punica granatum* extract (10% and 20%) and silver sulfadiazine group compared to control group. The mean period of epithelization was significantly reduced in topical *Punica granatum* extract (10% and 20%) group and silver sulfadiazine group as compared to the control.

**Conclusion:** *Punica granatum* peel extract is effective in healing burn wound and the effect was comparable to the standard drug silver sulfadiazine.

**Keywords:** *Punica granatum*, burn wound, epithelization period, wound contraction

**INTRODUCTION**

The burn injury is the one of the most common cause for the causality visits in a hospital and remains the major global concern. Of the burn injuries, majority of them are minor and thus can be managed on outpatient basis (Brigham, et al., 1996). Three zones of injury exist around a burn: a zone of necrosis, a zone of stasis, and a zone of hyperemia (Jackson DM, 1953).

There is a common practice regarding the use of skin disinfectants (ex. Betadine) in case of burn injury, but they have actually shown to delay the burn wound healing process and hence are discouraged (Baxter CR, 1993). Topical antibiotics are quiet effective in increasing the healing process in burn wounds. Currently Silver sulfadiazine is one of the commonly used agents for this purpose.

*Punica granatum* as such bears long history for medicinal use. The various parts of this plant have been used in traditional medicine and also have been stated in Egyptian Papyrus of Ebers (1550 BC). Many studies have shown roots to have effective anthelmintic properties (Caius J.F., 1923; Kim N.D., 1974; Kiuchi F, et al., 1989)). It is known to have anti microbial property against *Salmonella typhi* (Pérez, C., 1994) and *Vibrio cholerae* (Guivara J.M., 1994), the parasite *Giardia* (Ponce-Macotela M., 1994) and as well as amoeba and includes some viruses too (Zhang J., 1995; Stewart G., 1995).

In particular the peel extract of *Punica granatum* has extensively been studied for its antioxidant activity (Thring T, et al., 2009), cytotoxic activity (Kulkarni AP, et al., 2007), hypoglycemic activity (Hontecillas R, et al., 2009), hepatoprotective activity (Murthy KN, et al., 2002) and antiinflammatory activity (Julie J, et al., 2008).

We intend to find an alternative, safe, cheap and acceptable agent to enhance the burn healing process. Considering above discussed facts, the burn wound healing potential of topical aqueous extract of *Punica granatum* peel has been investigated on burn wound models in albino rats.

## OBJECTIVES

- To study the effect of a topical *Punica granatum* peel extract on healing of burn wound.
- To compare its effect with that of standard (Silver sulfadiazine).

## MATERIALS AND METHODS

**Animals:** Singly housed male Wistar rats (150-200g) were used in this study. They were given water ad libitum and fed with commercial food pellets. The study protocol was approved by institutional animal ethics committee, Kasturba medical college, Manipal (IAEC/KMC/20/2008-09) and care of the animals was taken as per standard guidelines. Animals will be housed individually in polypropylene cages containing sterile paddy husk as bedding throughout the study and will have free access to sterile food (animal chow).

**Method of aqueous extract preparation:** The dried coarse pieces of pomegranate peel were obtained from local ayurvedic shop and authenticated by a Botanist. The particles were finely powdered followed by filtration to remove large sized remnants. The separated powder was transferred to a round bottom flask and 1.5 litres of distilled water was added and soaked for 2 hours followed by boiling for 3-4 hours. The procedure was repeated twice to obtain the liquid extract. The clear supernatant was evaporated on a water bath and the remaining liquid was dried in a desiccator to obtain a thick paste (yield 17%).

## Study design and Dosing Schedule

*Punica granatum* aqueous peel extract and silver sulphadiazine will be administered topically, twice daily from day 0 to day of complete healing or the 21st postoperative whichever occurred earlier, in the partial thickness burn wound. There were four groups in the study viz. control (topical petroleum jelly), standard (topical Silver Sulphadiazine Cream) and test groups (topical *Punica granatum* Peel Extract 10% and 20 %).

## PROCEDURE

Partial thickness burn wounds are inflicted upon wistar rats starved overnight and under ketamine (dose- 50mg/kg ip), by pouring hot molten wax at 80°C in to a metal cylinder with 300mm square circular opening, placed on the back of the animal. Ringer lactate (1ml/kg) was administered i.p., immediately after injury and on subsequent days. The parameters to be observed in the study are as follows

## Epithelization period

It is monitored by noting the number of days required for the eschar to fall off from the burn wound surface without leaving a raw wound behind.

## Wound contraction

It was noted by following the progressive changes in wound area planimetrically, excluding the day of wounding. The size of the wound was traced on a transparent paper every two days, throughout the monitoring period. The tracing was then transferred to 1 mm square graph sheet, from which wound surface is then evaluated. The evaluated surface area was then employed to calculate the percentage of wound contraction, taking the initial size of the wound, 300 mm square, as 100% by using the following equation.

$$\text{Percentage of wound Contraction} = \frac{\text{Initial day wound size} - \text{Specific day wound Size}}{\text{Initial day wound size}} \times 100$$

**Statistical analysis:** Results were expressed in Mean±SD and were analyzed by one way analysis of variance (ANOVA) and post hoc analysis was done using Tukey's using SPSS package version 16. P-value < 0.05 was considered to be significant.

## RESULTS & DISCUSSION

The percentage of wound contraction was significantly increased in the topical *Punica granatum* extract (10% and 20%) and silver sulfadiazine group as compared to control group. There was no significant difference in the percentage of wound contraction when one test group was compared with another test group. Percentage of wound contraction on in plant extract and silver sulfadiazine group is significant, on

**TABLE 1-** Effect of Topical aqueous extract of *Punica granatum* peel on burn wound models in albino rats. (Values expressed in Mean±SD) Wound Contraction (%)

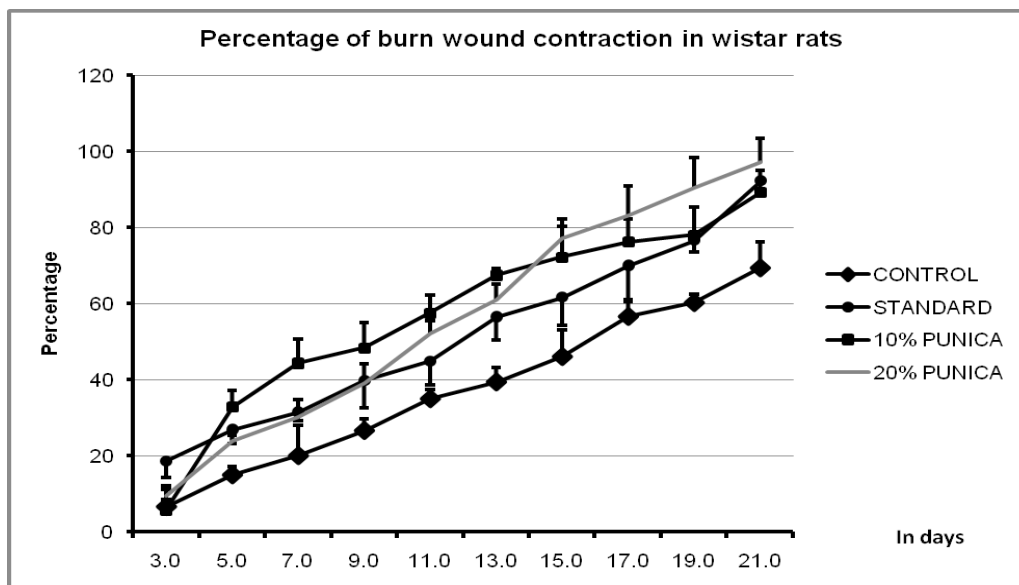
Groups	5 <sup>th</sup> day	9 <sup>th</sup> day	13 <sup>th</sup> day	15 <sup>th</sup> day	17 <sup>th</sup> day	19 <sup>th</sup> day	21 <sup>th</sup> day
Control	14.99±2.15	26.67±3.01	39.47±3.7	46.15±6.9	56.7±3.84	60.35±2.21	69.5±6.79
Silver sulfadiazine	27±3.71	39.9±7.21	56.5*±6.2	61.7*±7.4	70.6*±8.9	76.7*±3.18	92.2*±4.0
10% Punica extract	32.9*±4.33	48.4*±6.62	67.5*±1.7	72.3*±7.9	76.3*±6.0	78.1*±7.41	89.3*±5.6
20% Punica extract	24.1±1.4	39.1*±5.21	61.1*±4.0	77.3*±5.0	83.3*±7.5	90.6*±7.84	97.3*±6.2

N= number of rats in each group= 6; \* P-value <0.05, significant in comparison to control (ANOVA followed by tukey's

**Table 2 -** Effect of Topical aqueous extract of *Punica granatum* peel on epithelization. (Values expressed in Mean±SD)

Groups	Epithelization Period (In Days)
Group 1 - Control (Petroleum Jelly)	27±2.34
Group 2 - Standard (Silver Sulphadiazine)	22±2.11*
Group 3 - 10% Punica granatum Peel Extract	23±3.11*
Group 4 - 20% Punica granatum Peel Extract	21±1.98*

N= number of rats in each group= 6; \* P-value <0.05, significant in comparison to control (ANOVA followed by tukey's post hoc analysis



13, 15, 17, 19 and 21<sup>st</sup> day, as compared to control (Table 1, Graph 1). The mean period of epithelization was significantly reduced in topical *Punica granatum* extract (10% and 20%) group and silver sulfadiazine group as compared to the control (Table 2).

Topical gel application of alcoholic extract of peel has shown beneficial healing effect in rat excision wound model (Murthy KN, et al., 2004). In our previous study, oral administration of aqueous *Punica granatum* peel extract was also found to have good wound healing activity in dexamethasone suppressed incision, excision and dead space wound models in wistar rats (Shalini Adiga, et al., 2010). The present study also shows increased wound healing effect in burn wound model. These effects could be attributed to its anti-oxidant property (Thring T, et al., 2009). In one of the invitro study, *Punica granatum* extract has shown to be effective against strains of *Staphylococcus aureus* and MRSA but was not effective against *Pseudomonas aeruginosa* (Poth T, et al., 2007). Hence, aqueous *Punica granatum* peel extract can be good, inexpensive and effective alternative to silver sulfadiazine.

## Conclusion

In this study, *Punica granatum* peel extract showed significant burn wound healing property as compared to control (petroleum jelly) and the effect was comparable to the standard drug silver sulfadiazine. *Punica granatum* peel extract can play important role in burn patients.

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