Research Article

EFFECT OF *PUNICA GRANATUM* PEEL AQUEOUS EXTRACT ON NORMAL AND DEXAMETHASONE SUPPRESSED WOUND HEALING IN WISTAR RATS

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ABSTRACT

Pomegranate (*Punica granatum*) is an ancient fruit used in various systems of medicine for a variety of ailments. Objective of the study was to determine wound healing effect of aqueous extract of *Punica granatum* peel on normal and dexamethasone suppressed wounds in wistar rats. Three wound models viz. incision, excision and dead space wounds were used in this study. The parameters studied were breaking strength in case of incision wounds, epithelialization and wound contraction in case of excision wound and granulation tissue dry weight, breaking strength and hydroxyproline content in case of dead space wound. Aqueous extract of *Punica granatum* peel treated groups (100 mg/kg and 50 mg/kg) showed significant ($P<0.05$) improvement in incision and granulation tissue breaking strength and hydroxylproline content as compared to control. Co administration of aqueous extract of *Punica granatum* peel at 100 mg/kg with dexamethasone had significantly ($P<0.05$) improved the incision wound, granulation tissue breaking strength and hydroxyproline content as compared to dexamethasone alone treated group. There was significant decrease ($P<0.05$) in epithelization period and significant improvement in percentage of wound contraction with aqueous extract of *Punica granatum* peel as compared to control group and has effectively reversed the effects of dexamethasone ($p<0.05$). In this study, aqueous extract of *Punica granatum* peel has shown to posses wound healing property and it effectively reverses the dexamethasone suppressed wound healing in all the three wound models in wistar rats.

Keywords: *Punica granatum*, wound healing, dexamethasone.

INTRODUCTION

Wound healing is a complex process characterized by homeostasis, reepithelisation, granulation tissue formation and remodeling of the extracellular matrix. Reports about medicinal plants affecting various phases of wound healing process are abundant in scientific literature[1]. Pomegranate is an ancient fruit used in various systems of medicine for a variety of ailments. Dried fruit peel (pericarp) is used for diarrhea and to treat respiratory infections. The fruit peel exerts diverse pharmacological functions such as antioxidant activity[2], cytotoxic activity[3], hypoglycemic activity[4], hepatoprotective activity[5] and antiinflamatory activity[6]. Pomegranate peel aqueous extract has shown potent dermal effect on cultures of human skin cells, stimulating dermal fibroblast proliferation and collagen synthesis while inhibiting major collagen degrading enzyme[7]. Topical gel application of alcoholic extract of peel has shown beneficial healing effect in rat excision wound model.[8]

Glucocorticoids are known to suppress wound healing. Dexamethasone is a very potent antiinflamatory glucocorticoid used in organ transplantation and skin allografts. Dexamethasone strongly interferes with both the synthesis and degradation of collagen[9]. There are not many agents which are able to successfully overcome the anti-healing effect of corticosteroids. On the basis of the above discussed facts, the wound healing potential of orally ingested aqueous extract of *Punica granatum* peel has been investigated for its healing effect on different wound models in albino rats when used alone and in presence of dexamethasone induced suppression of wound healing in rats.

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, KMC Manipal (IAEC/KMC/20/2008-09) and care of the animals was taken as per standard guidelines Following overnight starving, animals were anesthetized with ketamine (50mg/kg body weight) and suitably wounded after shaving the area to be operated to bear incision, excision and dead space wounds. The wounds were not dressed and no systemic or local antimicrobial agents were used.

Method of aqueous extract preparation: The dried coarse pieces of pomegranate peel were obtained from local ayurvedic shop and authenticity confirmed by a phytochemist. The particles were finely powdered in a mixture 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%).

Acute toxicity study: Five groups of 2 rats each were used for the acute toxicity study. The animals were fasted for

24 hours prior to drug treatment. Rats were given 10mg/kg, of extract orally initially. The animals were observed for gross behavioral changes frequently for the next four hours and then after 24 and 48 hours for any toxic effect[10]. The subsequent doses were then increased by a factor of 1.5 on a log scale. The drug produced no mortality up to 1000 mg/kg dose. However, signs of apathy and mental dullness were observed during first 24 hours of observation at 1000 mg/kg. The doses for main study were selected as 100 mg/kg body weight and 50 mg/kg body weight based on the acute toxicity studies and doses used in previous studies [11].

Wound Models

Dead Space Wounds: Dead Space Wounds were created by implantation of a polypropylene tube (2.5x0.5cm) beneath the dorsal paravertebral lumbar skin. On day 10 the harvested granulation tissue was subjected to physical as well as biochemical evaluation. Hydroxyproline was estimated calorimetrically[12] and breaking strength of granulation tissue was measured by continuous water flow technique[13].

Sutured incision wounds: Two para vertebral straight incisions of 6cm each were made through the entire thickness of skin one on either side of at least 1cm lateral to vertebral column as per the method of Ehrlich and Hunt[14]. Wounds were closed with interrupted sutures, 1cm apart. The sutures were removed on 7th day. Wound breaking strength was measured on 10th post wounding day.

Excision Wounds: Excision wounds were created by excising a circular piece (500mm^3 in area) of full thickness skin from the dorsal interscapular region[15]. Wound contraction was monitored by measuring wound area, planimetrically, on alternate days till the wounds were completely healed. This was expressed as percentage of wound contraction. Time taken for complete epithelialisation was noted by recording the days required for fall of scab leaving no raw wound behind.

Drug administration: Animals bearing a given wound were divided into 5 groups of 6 animals each. First group of animals received distilled water and served as control, Second group received 50mg/kg, third group received 100mg/kg of aqueous extract of *Punica granatum* peel orally. Fourth group received dexamethasone (0.17mg/kg im) and the fifth group received dexamethasone (0.17mg/kg im) with aqueous extract of *Punica granatum* peel 100mg/kg extract orally.

The *Punica granatum* peel extract was given daily, dexamethasone on alternate days from day 0 to the day of complete healing or the 10th post-operative day, according to the wound healing model.

Statistical analysis: Results were analysed by one way analysis of variance (ANOVA) using post hoc Tukey's test and p-value < 0.05 was considered significant.

RESULTS AND DISCUSSION

In dead space wound model the aqueous extract treated animals showed significant increase in levels of hydroxyproline content, dry granulation tissue weight and granulation tissue breaking strength. The dexamethasone treated animals depressed wound healing as evidenced by significant decrease (p-value < 0.05) in breaking strength, hydroxyproline content and granulation tissue. In the same wound model administration of peel extract of *Punica granatum* reversed the antihealing effect of dexamethasone (table1).

In the incision wound model a significant increase (p-value < 0.05) in wound breaking strength in the extract treated group was observed as compared to control group. A significant decrease in wound breaking strength in dexamethasone alone treated group was observed as compared to control group. Suppression of wound breaking strength by dexamethasone was effectively reversed (p-value < 0.05) when treated along with aqueous extract of *Punica granatum* peel 100mg/kg extract as shown in table 1.

Table 1: Effect of aqueous extract of punica granatum peel in incision and dead space wound model

Groups	Incision wound breaking strength(g)	Dead space wound healing parameters		
		Dry tissue weight (mg/100g rat)	Breaking strength (g)	Hydroxyproline mg/g tissue
Control(distilled water)	208.08±11.12	35.16±2.31	236.16±3.43	19.16±1.47
punica (50mg/kg)	237.83±8.6*	40.5±1.87*	242.33±3.26*	22.66±1.75*
Punica (100mg/kg)	263.33±10.79*	45.16±2.31*	245.83±2.31*	26.33±2.16*
Dexa(0.17mg/kg)	106.75±10.78	44.66±2.16	139.5±8.04	15.5±1.37
Dexa(0.17mg/kg)+ Punica(100mg/kg)	153.08±27.45#	40.33±1.63#	234.33±12.3#	19.66±1.21#

p-value <0.05 is significant; * significant vs control; # significant vs dexamethasone , using ANOVA post hoc by tukey's (Values are mean ±SE of 6 rats)

Table 2: Effect of punica granatum on wound contraction in an excision wound model

Groups	4th day	8th day	12th day	16thday	Epithelization period
Control(distilled water)	27±1.41	43.5±1.04	58±2.36	67.16±1.47	26.50±2.04
punica (50mg/kg)	27.66±2.33	47.83±0.98*	67.83±1.16*	82±1.41*	19.66±3.21*
Punica (100mg/kg)	28.33±1.21	48.33±0.81*	70±1.41*	83.83±1.16*	18.66±2.21*
Dexa(0.17mg/kg)	20.66±1.36	38.5±1.04	55.83±1.94	63.66±1.03	33.83±4.47
Dexa(0.17mg/kg)+ Punica(100mg/kg)	26.16±1.47#	43.16±1.47#	57.83±0.75	67.83±1.16#	26.33±3.03#

p-value <0.05 is significant; * significant vs control; # significant vs dexamethasone , using ANOVA post hoc by tukey's (Values are mean ± SE of 6 no. of rats)

In excision wound model the extract treated animals showed significant decrease (p-value < 0.05) in epithelization period (table 2) and significant increase in percentage wound contraction as compared to control. In dexamethasone alone treated group significant increase (p-value < 0.05) in epithelisation period and decrease in percentage wound contraction were observed when compared to control. These effects were reversed (p-value < 0.05) when treated with aqueous extract of *Punica granatum* peel 100mg/kg.

The complex process of healing involves various phenomena like wound contraction, granuloma formation, collagen maturation etc. The contribution for healing of such processes depends on the type of wound. Wound contraction plays a significant role in healing of excision wound while granuloma formation plays a role in healing of sutured incision and dead space wounds. The results of the present study clearly demonstrate that the aqueous extract of *Punica granatum* possess a definite prohealing action in normal healing as well as in the steroid depressed wound healing. An increase in wound breaking strength and hydroxyproline content of treated wounds may be due to increase in collagen concentration and stabilization of collagen fibres. Collagen the major component which supports and strengthens the extracellular tissue is composed of components like aminoacid and hydroxyproline, which has been used as a biochemical marker for tissue collagen[16]. Increase in dry granulation tissue weight indicates high protein concentration and collagen bundle formation.

In recent years oxidative stress has been implicated in a variety of degenerative process and disease. These include acute and chronic inflammatory conditions such as wound healing[17]. Pomegranates are a source of polyphenols and other antioxidants[18] pomegranate peel extract has markedly higher antioxidant capacity than the pulp extract in scavenging superoxide anion, hydroxyl and peroxyl radicals. The contents of total phenolics, flavinoids and protanthrocyaanidens are known to be higher in peel extract than pulp extract [19]. The free radical scavenging activity of plant phenolics and flavanoids help in wound healing[20]This could be the reason for the prowound healing activity of *Punica granatum* peel extract. The exact phytochemical component of the extract responsible for this effect however was not investigated. The various bioactive pomegranate fractions singly or in combination are to be identified to find its therapeutic potential use in the management of wound healing.

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