Wound healing potential of *Punica granatum linn* fruit peel.

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ABSTRACT

Objective: The present study was carried out to investigate the wound healing potential of the extract of dried peel of *Punica granatum L.* in albino wistar rat using various screening models.

Materials and methods: Fruit peel of *Punica granatum L* was collected, dried and extracted (maceration) using ethanol. Preliminary qualitative phytochemical screening was carried out. The in-vivo models were carried out i.e. excision, incision and dead space wound model using silver sulfadiazine as reference standard. **Results:** Dose dependent and significant ($p<0.05$) wound healing activity in *in-vivo models* were obtained. **Conclusion:** The result revealed promising wound healing potential of the plant. However further pharmacological investigation using isolated active ingredients can be carried out to confirm its efficacy and mechanism of action.

Keywords: *Punica granatum L*, excision, incision and dead space wound model

1. INTRODUCTION

Wound healing is the combination of regeneration and repair. Healing is said to be a characteristic wonder and wound healing is a vital condition and furthermore limit necessity of previous medications like anti-infection agents and furthermore their results. Restorative plants have been utilized all through human presence. In our laboratory investigation, we have found that the leaves of *Psidium guajava* extract showed wound healing potential in rats using various screening models of wound healing activity [1]. Herbs have been utilized

by numerous people of various traditions, way of life, foundation and propensities all through the past. [2]. WHO (world health organisation) has approximated that the 80% of in excess of 4000 million populace of the world rely upon conventional solutions for their prosperity and necessities and it can be accepted that the first piece of customary treatment includes the utilization of plant extricate as their dynamic standards [3,4,5,6].

PROCESS OF WOUND HEALING [7,8,9,10].

Clearly injuring process occurs in each organ and tissues of the body. Despite the fact that procedure of mending is ceaseless, in light of its physiological procedure that is going on in and around encompassing tissues, it is separated haphazardly into different after stages.

There are diverse procedures of intense tissue repair that has been initiated by the tissue harm; this may be taken after into four time-subordinate portions.

I. Coagulation and Haemostasis: After the damage momentarily initiate.

II. Inflammation: Subsequently begins in a minute.

III. Proliferation: It covers a main procedure of healing and it starts inside days of damage.

IV. Remodelling of wound: improvement of tissue scars happen which may last up to a year or more.

The pomegranate belongs to the family *Lythraceae.* The pomegranate fruit is typically in season in the Northern hemisphere from September to February, and the southern hemisphere from March to May. [11,12]. An attempt was made to investigate wound healing capability of concentrate of dried peel of *Punica granatum L.*

2. MATERIALS AND METHODS

2.1. Chemicals

Analgesic ether, Silver sulfadiazine were bought from Narsons Pharma, Chittoor, Andra Pradesh, India and Virchow Biotech (P) Limited.

2.2. ANIMALS

Wistar rats of both sex, weighing between 180-200g, 5 months old was procured from the animal house of NGSM institute of Pharmaceutical sciences, Deralakatte, Mangalore. The protocol of the examination was endorsed by Institutional Ethics Committee for animal experimentation NGSM institute of Pharmaceutical sciences. The rats were isolated into specific gatherings and kept in proper cages independently. Animals were appropriately balanced out for a week and all around kept up in standard condition at room temperature of 25± 2ºc with amend relative mugginess and also light and dim cycle of 12hrs. Nourishment and water was given to animals with standard dry pellet consume less calories, not indispensable over the span of the examination. All the exploratory methods were done in light of the CPCSEA rules, New Delhi and IAEC rules.

2.3 PLANT MATERIAL

The peel of *Punica granatum* fruit peel was gathered in the month of July 2017. The plant was identified by Dr. K V Nagalaxmamma, Associate Professor, HOD, Department of Botany, St.Aloysius school (Autonomus), Mangaluru. A sample of the fruit peel was submitted to the institutional herbarium and the sample No. is 16PYO12.

2.3. PLANT EXTRACTION

The peels of pomegranate were gathered and were cleaned from dirt and different adhering materials, washed and dried under shade for 2 weeks. The dried peels were smashed and after that it was subjected to maceration with the 90% of ethanol for 7 days with periodic blending in an extraction chamber. The substance was sifted by utilizing muslin material. The syrup got were dried on the water bath until the point that the dry concentrate was acquired and it was dull dark colour in shade in the wake of drying and was placed in the desiccator for further use.

2.4. PRELIMINARY QUALITATIVE PHYTOCHEMICAL INVESTIGATION

The ethanolic extract of *Punica granatum* fruit peel were subjected to qualitative examination for different phytoconstituents like alkaloids, carbohydrates, flavonoids, proteins, saponins, terpenoids, glycosides, phenol and steroids by using standard methods.

2.5. Screening of Wound healing activity of ethanolic extract of peel of *Punica granatum* by *In-vivo* technique:

2.5.1. Excision wound model method [1] :

Table1. Materials and methods for excision wound model

Animals	Albino wistar strain rats
Age and sex	Male
Body weight	180-200g
No of animals in each group	n=6
Number of groups	5
Route of administration	Oral route
Water and food	*ad.Libitum*

Table2. Treatment protocol for excision wound model:

Groups	Treatment
Group I (Control)	No drug treatment
Group II (Standard drug)	Silver sulfadiazine ointment (5%w/w)
Group III (Test)	Extract of 100mg/kg
Group IV (Test)	Extract of 200mg/kg
Group V (Test)	Extract of 400mg/kg

Procedure: Male rats weighing 180-200g was taken and anesthetized with 1ml of ketamine hydrochloride (i.p 10mg/kg body weight) and the dorsal surface of the animal was shaved appropriately and the territory in which the injury must be made was checked utilizing indelible marker and the injury of circular area 4.9 cm^2 and profundity of 0.2cm was finished utilizing surgical cutting edge, toothed forceps and pointed sharp scissors and whole wound was left open. Not long after the making of the wound the group I animals were kept as control. Group II was the reference standard and was treated with silver sulfadiazine ointment and was topically connected constantly and the Group III, IV, V animals belonged to the test group and received 100mg/kg, 200mg/kg and 400mg/kg of ethanolic extract of dried peel of *Punica granatum*. The wound area was checked simultaneously on 1^{st}, 4^{th}, 8^{th}, 11^{th} and 14^{th} days by tracing the wound over the transparent paper and area was measured using $1mm^2$ graph paper. Change in the wound area was observed and wound contraction was evaluated by using formula.

$$\%\text{wound contraction} = \left(\frac{\text{Healed area}}{\text{Total wound area}} \times 100 \right)$$

Healed area = Original wound area - Present wound area

2.5.2. Incision wound model method [13]:

Procedure: Male rats weighing 180-200g were taken and anesthetized with 1ml of ketamine hydrochloride (i.p 10mg/kg body weight) and the dorsal surface of the animal was shaved legitimately and a longitudinal paravertebral entry point was done around 6 cm long utilizing surgical sharp edge however skin and cutaneous tissue on the back. Once the long entry point was done the separated skin was sutured utilizing surgical string and bended needle around 1cm separated. The injury was left open and stripped. Group I was untreated and kept as control.

Group II was the reference standard and was treated with silver sulfadiazine ointment and were topically connected once in a day.

Group III, IV, V were the test group of ethanolic concentrate of *Punica granatum* L. organic product peel 100mg/kg, 200mg/kg and 400mg/kg were administrated orally to every one of the animal individually. The sutures were opened on the 8th day post injuring in the anesthetized condition. The skin breaking strength was seen on the tenth day. Breaking strength was estimated utilizing tensiometer. The anesthetized animal was kept on the table and line was drawn on the both side of the injury 3mm away. With the assistance of forceps the line was grasped at the each end restricted to each other. One of the forceps was solidly upheld and the other was associated with the unreservedly suspended metal plate. At the point when the weight was added to the plate because of progressive increment in the weight there was steady pulling separated the injury edges once the injury opens the including of the weight stops. Option of weight which was included was used to assess the breaking strength in grams.

2.5.3. Dead space wound model method [14] :

Procedure: Male rats weighing 180-200g was taken and anesthetized with 1ml of ketamine hydrochloride (i.p 10mg/kg body weight) and the dorsal surface of the rat was shaved

appropriately and an entry point wound was made and embedding the disinfected cotton pellets of weight 10mg were embedded on the lumbar area on the dorsal surface of the rodent. The injury was left uncovered. Group I was untreated and kept as control. Group II was the reference standard and was treated with silver sulfadiazine salve and was topically applied constantly and the Group III, IV, V was the test group of ethanolic extract of *Punica granatum* peel 100mg/kg, 200mg/kg and 400mg/kg was administered orally. On the tenth post injuring day granulation tissue collected on the embedded cotton pellets was acquired and the wet weight of the granulation tissue was assessed. These granulation tissue was dried and weighed and the dried weight of the granulation tissue was observed.

2.6. Statistical Analysis

All the data will be expressed in the form Mean ± SEM and those will be analyzed by one way analysis of variance (ANOVA) which were followed by the post hoc scheffe's test using SPSS computer software version 10. P value less than 0.05 were taken as statistically significant.

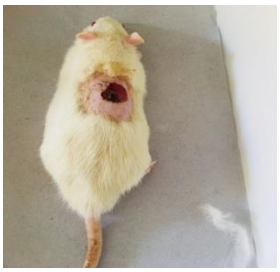
3. RESULTS

Excision wound model

Table no.3. Effect of ethanolic extract of *Punica granatum* L. fruit peel on excision wound model.

Parameter	No. of days	Control	Standard	100mg/kg extract	200mg/kg extract	400mg/kg extract
Wound area in mm^2	1st	492.0±0.8	491.3±1.4	487.1±1.6	488.8±1.0	489.8±1.8
	4th	406.3±2.0^{b}	279.6±1.7^{a}	399.6±0.9^{b}	393.6±1.3^{a,b}	382.6±1.5^{a,b}
	8th	327.0±1.4^{b}	191.3±1.7^{a}	314.1±1.6^{a,b}	204.6±1.0^{a,b}	201.0±1.7$^{a.b}$
	12th	194.0±1.6^{b}	67.3±0.8^{a}	153.5±2.1^{a,b}	100.8±1.0^{a,b}	95.1±1.5^{a,b}
	14th	150.1±3.3^{b}	35.5±0.8^{a}	94.5±1.7^{a,b}	52.5±1.6^{a,b}	45.6±1.3^{a}
% contraction	4th	17.41±0.2^{b}	43.08±0.2^{a}	18.0±0.2^{b}	19.3±0.3^{a,b}	21.8±0.3^{a,b}
	8th	33.5±0.17^{b}	61.06±0.2^{a}	35.5±0.2^{a,b}	58.0±0.2^{a,b}	58.9±0.2^{a,b}
	12th	60.5± 0.27^{b}	86.2±0.13^{a}	68.4±0.37^{a,b}	79.3±0.17^{a,b}	80.5±0.2^{a,b}
	14th	69.11±0.11^{b}	92.7±0.15^{a}	80.6±0.30^{a,b}	89.2±0.33^{a,b}	90.6±0.2^{a,b}

The values are expressed as mean ±SEM (n=6) $p < 0.05$ is considered as statistically significant. a=$p<0.05$ when compared to control group, b=$p< 0.05$ when compared to standard group


4th DAY 8th DAY 12th DAY

15thDAY

Figure.2. Wound healing activity by excision model (400mg/kg extarct on 4th, 8th, 12th, 15th day).

In the present study it was found that ethanolic extract of *Punica granatum* L. fruit peel increased the percentage of wound contraction. Animals treated with 400mg/kg showed percentage wound contraction of 90.6% and that of control animals 69.1% by the16th day. The standard drug treated animalsshowed the maximum % of wound contraction ie. 92.7. Hence the extract of 400mg/kg showed better activity compared to 100mg/kg and 200mg/kg as well as control. The dose of 400mg/kg extract and standard drug showed almost similar activity.

- **Incision wound model**

Table no.4. Effect of ethanolic extract of *Punica granatum* L. fruit peel on incision wound model.

Parameter	Control	Standard	100mg/kg extract	200mg/kg extract	400mg/kg extract
Wound breaking strength	296.3± 10.2[b]	431.6 ±10.7[a]	323.3±4.4[b]	381.6 ±7.3[a,b]	422.5± 11.5[a]

The values are expressed as mean ±SEM (n=6) $p < 0.05$ is considered as statistically significant.

a=p<0.05 when compared to control group,

b=p< 0.05 when compared to standard group.

The tensile strength of the extract treated group was found to be (422.5± 11.5) which was higher than that of control treated group (296.3± 10.2) of animals and slightly lesser than that of standard treated group (431.6 ±10.7) of animals on 10th post wounding day.

Dead space wound model

Table no.5: Effect of ethanolic extract of *Punica granatum* L. fruit peel on dead space wound model**..**

Parameter	Control	Standard	100mg/kg extract	200mg/kg extract	400mg/kg extract
Wet weight	312±1.06	482.8±1.7	418.6± 1.45	436.3± 1.28	470.3± 0.6
Dry weight	91± 1.0	167± 2.0	113 ±1.6	137 ±1.6	151± 0.9

The values are expressed as mean ±SEM (n=6) . a=p<0.05 when compared to control group, b=p< 0.05 when compared to standard

From the study the data obtained were, the wet weight of extract, control and standard treated animals were found to be 470.3± 0.6, 312±1.06 and 482.8±1.7 respectively. And the dry weight of sample extract, control and standard treated animals were found to be 151± 0.9 , 91± 1.0 and 167± 2.0 respectively (The data is depicted in table no 18).

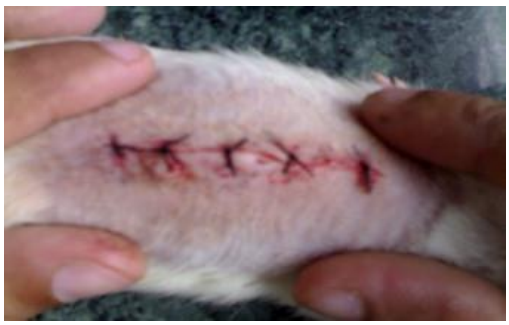


Figure.3. Incision wound model

4. DISCUSSION

4.1 Wound healing activity[15]:

Wound healing activity is an outrageous complex marvel including various very much orchestrated forms, including recovery of parenchyma cells, relocation and multiplication of both parenchymal and connective cells, blend of extracellular lattice protein, remodelling of connective tissue parenchymal segments, collagenisation and fulfilment of wound strength. The wound healing process continues normally, as the harmed tissue endeavours to re-

establish homeostasis. Notwithstanding, the hazard factors, for example, diseases and over the top fiery response may trade off the repair procedure.

4.1.1. Excision wound model:

In excision wound model, the wound healing potential of ethanolic extract was studied in terms of reduced wound area, causing wound contraction. Oral administration of *Punica granatum* L. fruit peel extract increased the percentage of wound contraction and completed wound healing by 16th day indicating rapid epithelisation and collagenation.

4.1.2. Incision wound model[16]:

The wound healing property of plant extract can also be determined by measuring tensile strength of incision wound by tensiometer. The wound healing agents have the property to increase the deposition of collagen content, which provides strength to the tissues and forms cross-linkages between collagen fibres. From the result it is evident that the extract was able to increase breaking strength of the tissue covering the wound. This may be due to enhanced collagen formation.

4.1.3. Dead space wound model[17] :

The effect of oral administration of the extract *Punica granatum* L. fruit peel treated group and control treated group on dead space wound model was assessed by increase in the weight of granulation tissue and increase tensile strength. The collagen maturation improved by increased cross-linking of collagen fibres. The increased weight of the granulation tissue also indicated the presence of higher protein contents. The extract of *Punica granatum* L. fruit peel also showed significant activity when compared to standard (sliver sulphadiazene ointment).

5. CONCLUSION

The study demonstrated that the ethanolic extract of dried peel of *Punica granatum L* fruit has appropriate properties to hasten wound repairing action. The wound healing potential may be due free radical scavenging activity and thus enabling rapid maturation of granulation tissue. [18] However studies at molecular levels involving effect on chemical mediator levels might be needed to elucidate the mechanism of action of *Punica granatum* fruit peel as a wound healing agent.

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