



Research Article

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Punica granatum Rind, a Traditional Herbal Medicine: Effect on wound healing

Nouf Dughayshim Alshammari¹, Nudrat Fatima¹ and Naira Nayeem¹

¹College of Pharmacy, Northern Border University, Rafha, Kingdom of Saudi Arabia

ABSTRACT

The study was conducted to develop a cost effective and safe herbal medicine for open wounds and to prove on scientific lines the status of traditional herbal medicine used in Northern part of Saudi Arabia. Bed sore is an open wound formed on the skin, which is a common problem faced by the patients of limb paralysis and other long term bed ridden patients. In this research project a traditional herbal medicine, used in Northern part of Saudi Arabia for the treatment of severe condition of open wounds, is studied in male Wistar Albino rats weighing between 150-200g. For this study 12 male albino rats of Wistar strain were selected, weighed and divided into 3 groups comprising of 4 rats in each group. The circular wounds of 400mm² size were produced on the dorsal thoracic region following the reported method. Animals of Group 1 were treated with test drug (dried powder of *Punica granatum rind*), Group 2 with Fucidin ointment and Group 3 with Vaseline. The wounds were measured and application was made using standard method on alternate days. The animals were monitored daily for their health. The data was collected and statistically analyzed using student t-test considering $p < 0.05$ as significant. The test drug exhibited highly significant activity compared to others i.e. all animals of Group 1, 2 and 3 healed on 15, 17 and 21 days respectively. This may lead to formulation of a cost effective and safe herbal medicine for bed sore. TLC study was also conducted on extract of pomegranate rind which revealed the presence of rutin, quercetin, gallic acid and salicylic acid. The wound healing activity may be due to the presence of these phenolic compounds.

Keywords: *Punica granatum rind, wound healing, TLC, traditional herbal medicine*

INTRODUCTION

In this research, a traditional herbal medicine i.e. *Punica granatum rind* used in Northern part of Saudi Arabia for the treatment of severe condition of bed sore, is studied against open wounds in male Wistar Albino rats. The medicine is formulated with dried pomegranate rind which was powdered and mixed with Vaseline base in 10% w/w concentration.

In ancient Ayurvedic traditional medicine, the rind of the fruit and the bark of pomegranate tree are used in the treatment of diarrhea, dysentery, and intestinal parasites [Jindal and Sharma, 2004], for nose bleeds and gum bleeds, toning skin, and treating hemorrhoids [Manohar, 2002]. Pomegranate juice is employed as an eye drop to slow the development of cataracts [Vasant Lad, 2002]. Pomegranate seeds and rind are also contraceptive and abortifacient used as vaginal suppository [Riddle J, 1992].

The most abundant phytochemicals in pomegranate juice are polyphenols, including the hydrolyzable tannins or ellagitannins. These are formed when ellagic acid and/or gallic acid binds with a carbohydrate and are known as punicalagins. Compared to the pulp, the inedible pomegranate peel contains as much as three times the total amount of polyphenols [Singh et al, 2002], condensed tannins [Nasr et al, 1996], catechins, gallo catechins and prodelpinidins [Plumb et al, 2002]. Many researchers have reported antioxidant properties of Pomegranate [Chidambara Murthy, 2002; Negi et al, 2003; Li et al, 2006]. Punicalagins have shown free-radical scavenging properties in laboratory

experiments [Kulkarni et al, 2007] and are being studied for their potential biological activity in humans [Seerum et al, 2006; Mertens-Talcott et al, 2006]. It is also reported that it reduces common carotid intima-media thickness, systolic blood pressure and LDL oxidation [Aviram et al, 2001; Aviram et al, 2004]. Concentrated pomegranate juice improves lipid profiles in diabetic patients with hyperlipidemia [Esmailzadeh, 2004]. The present study was designed with an aim to develop a cost effective and safe herbal medicine for bed sore and to prove on scientific lines the use of *Punicagranatum* rind as a traditional herbal medicine used in Northern part of Saudi Arabia.

MATERIALS AND METHODS

Thin layer chromatographic studies: TLC was performed using precoated silica gel

plates 60F254. Different mobile phases were used and constituents were detected by observing and comparing the *R_f* value of the standard biomarkers with the spot on the TLC plates. The biomarkers used were flavonoids (rutin and quercetin) and phenolicacids (gallic acid and salicylic acid).

Preparation of Test Drug

Test material was prepared by drying pomegranate peel in sunshade, crushing to powder and preparing 10% ointment in Vaseline base.

Procurement of Test Animals

Twelve male albino healthy rats weighing between 150-200g of Wistar strain were selected from Animal House of College of Pharmacy, Rafha. The animals were kept in quarantine 1 week before the experiment. They were fed with standard rodent diet of pellets and provided water *ad libitum*. They were monitored daily for their health and untoward behavior. The experiment was conducted keeping in view the online guidelines by Committee on Animal Research and Ethics (CARE).

Grouping of Animals

The rats were divided into three groups comprising of 4 rats in each group. Group 1 was treated with test drug (dried powder of *Punica granatum* rind in Vaseline base), Group 2 with Fucidin ointment (manufactured by Leo Pharmaceuticals, Denmark) and Group 3 with blank Vaseline serving as negative control group.

Development of Wounds

The rats were shaved and circular wounds of approximately 400mm² size were produced on the dorsal thoracic region using sharp scissors after anesthesia with chloroform (Mahmood *et. al.*, 2005; Imran *et al*, 2015).

Measurement of Wound Size

The wounds were measured next day of wound creation and the reading served as initial reading. The wound area was recorded on transparent polythene sheets and counted using graph paper under sheet as described elsewhere (Girish and Patil, 2005).

Application of Drug

The application of test drug i.e. ointment of pomegranate peels, standard drug Fucidin and Vaseline base as control drug was done on Group 1, 2, and 3 respectively in the form of thin film covering the whole wound. The application was done on alternate days and recorded. The animals were monitored daily for their health and untoward behavior.

Statistical Calculation

The data was collected and statistical analysis was carried out using student *t*-test considering *p* value less than 0.05 as significant.

RESULT

Table 1: Wound healing size in mm²

S.No	Day	Test	Standard	Control
1	0	405.31 ± 25.55	415.31 ± 19.61	410.25 ± 20.05
2	3	347.06 ± 17.73	333.25 ± 18.50	343.00 ± 38.00
3	5	226.50 ± 43.85*	289.00 ± 0.000	289.50 ± 27.77
4	7	81.50 ± 14.71*	140.25 ± 41.28*	197.50 ± 41.02
5	9	17.31 ± 5.42	42.06 ± 17.89*	86.25 ± 17.33*
6	11	11.81 ± 4.96	19.56 ± 6.46	38.78 ± 2.58
7	13	1.03 ± 0.745 ¹	4.06 ± 3.51	6.72 ± 1.63
8	15	0.000 ± 0.000	1.42 ± 0.59 ²	4.00 ± 0.000
9	17	0.000 ± 0.000	0.000 ± 0.000	1.11 ± 0.22 ¹
10	19	0.000 ± 0.000	0.000 ± 0.000	0.25 ± 0.500 ³

All readings are mentioned in Mean ± SEM

50% Epithelization Time*

Number of Animals Healed ^{1, 2 and 3}

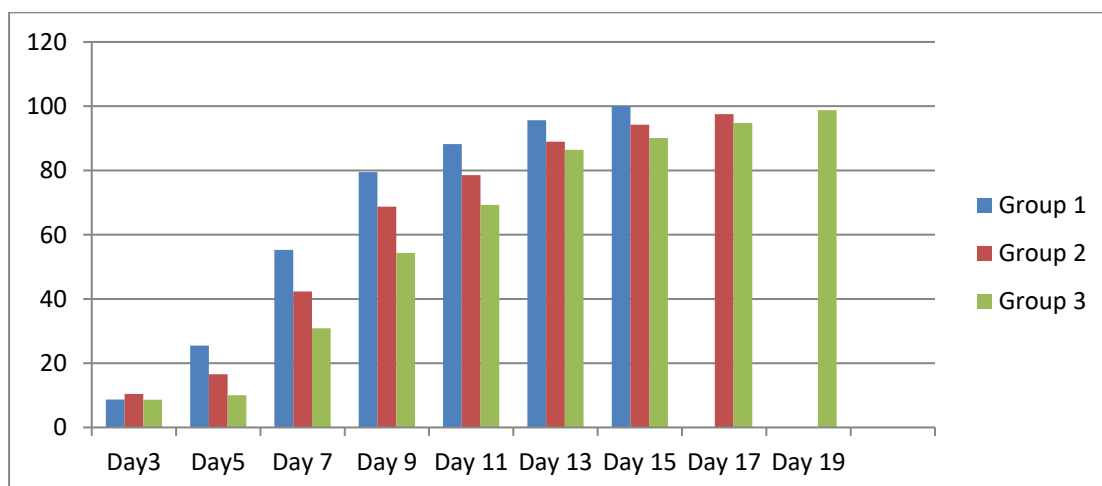
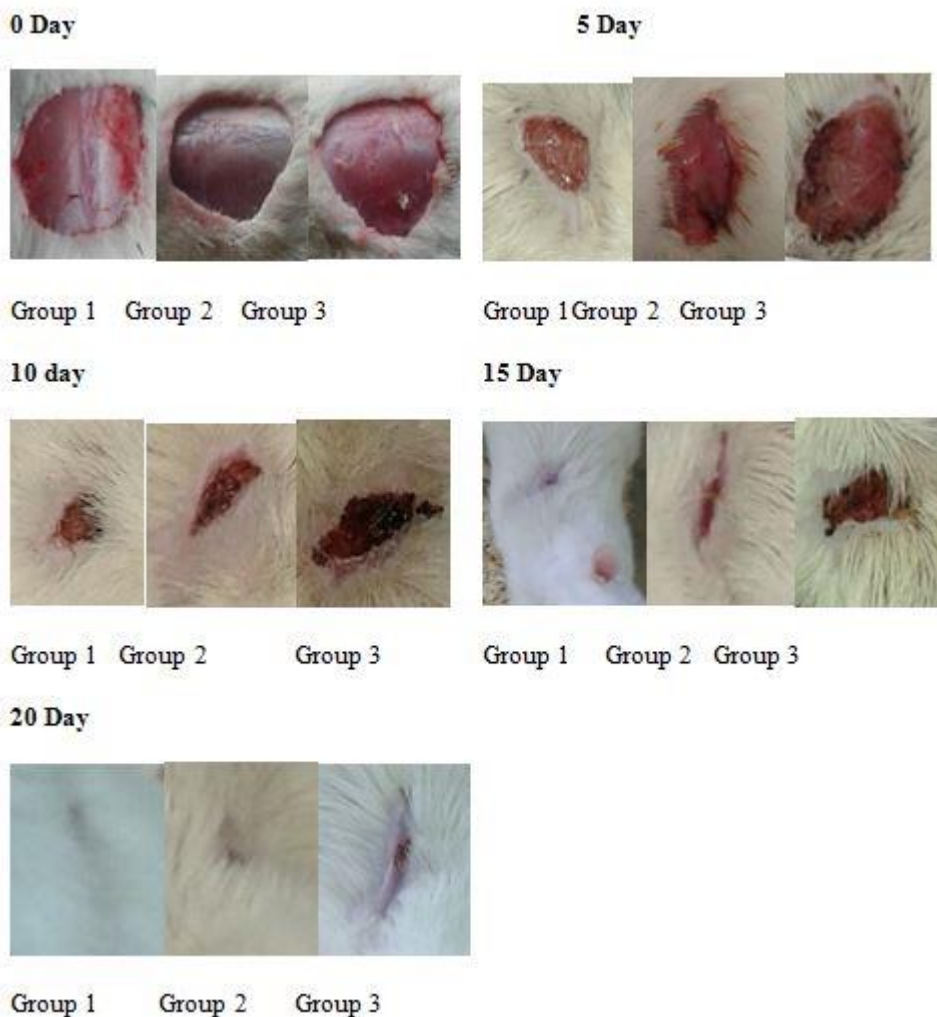


Figure 1: Comparison of % Healing between 1, 2 and 3 Groups



DISCUSSION

Herbal medicine still contributes seventy percent of world population as alternative treatment. Northern region of Saudi Arabia, though one of the oldest civilization, most of the area is rural and prefers herbal medicine.

It is mentioned in Holy Qur'an that pomegranates grow in the gardens of paradise and pomegranates three times as examples of good things Allah creates (**Surat Al-'An'am**).

It was found that till present no work is carried out on dried powder of pomegranate. Some scientific work is done on methanol and ethanol extracts pomegranate peels [Murthy et al, 2004; Nema et al, 2013; Ma et al, 2015] while others on aqueous extract [Shalini et al, 2010]. It is noted that sometimes there is difference in results exhibited by extract and dried part. In the literature search it was found that some researchers carried out wound healing activity in methanol and ethanol [Hemmati et al, 2014] activity of grape and others compared it with dried grape skin which were found to be different [Nayak et al, 2010]. Results of present study were found highly significant with respect to test drug i.e. wound size $9.000 \pm 0.816\text{mm}^2$ on 7th day, completely healed on 15th day (50% wound healing in 6 days, 100 % wound healing in 15 days) as compared to standard drug $11.750 \pm 0.816\text{mm}^2$ on 7th day, $1.175 \pm 0.236 \text{mm}^2$ on 15th day, completely healed on 17th day (50% wound healing in 8 days, 100 % wound healing in 15 days) and control $14.000 \pm 1.708\text{mm}^2$ on 7th day, $2.000 \pm 0.000\text{mm}^2$ on 15th day, completely healed on 21st day arranged in Table 1 (50% wound healing in 17 days, 100 % wound healing in 21 days as shown in figure1). It was found that dry powder promotes healing in less time. It is time saving and cost effective also. Another benefit is that it increases patient compliance as mentioned earlier that some old patients are psychologically disturbed with modern techniques and some do not agree to use modern medicine. Plant metabolites like phenolic acids and flavonoids have an active role in wound healing. TLC was performed and the results revealed the presence of rutin [Chloroform: Methanol (9:5)],

quercetin [Chloroform :Methanol (3:4)], gallic acid [Petroleum ether: Methanol (9:4.5)] and salicylic acid [Chloroform :Methanol(5:2)]. The wound healing properties of the peel may be attributed to the presence of these phenolic compounds.

CONCLUSION

The results of this study provide scientific proof of the traditional use of pomegranate peel for the treatment of bed sores in the rural areas of Northern Border Province. The formulation can be further subjected to stability studies and modified to suit the local population.

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