PharmacologyOnLine December 30, 2016

Archives • 2016 • vol.3 • 203-208

EVALUATION OF WOUND HEALING ACTIVITY OF HYDROALCOHOLIC EXTRACT OF BANANA (*MUSA ACUMINATA*) FRUIT'S PEEL IN RABBIT

Pari Tamri[1*], Aliasghar Hemmati[2], Ali Amirahmadi[3], Jaber Zafari[2], Babak Mohammadian[4], Mohammadshafi Dehghani[2], N allahyari[2]

[1]Department of pharmacology and toxicology, School of Pharmacy, Hamadan University of Medical Sciencesm, Hamadan, Iran
[2]School of Pharmacy, Toxicology Center, Jundishapur University of Medical Sciences, Ahvaz, Iran
[3]Department of health, Valiasr hospital research center, Rescue and treatment of police force, Tehran, Iran
[4]Department of Pathology, Faculty of Veterinary, Shahid Chamran University, Ahvaz, Iran

ptamri@gmail.com

Abstract

Banana (*Musa acuminata*) peel is a rich source of many nutrients and considered high in carbohydrates. It has been traditionaly used to treat diarrhea, anemia and ulcers. Some studies have shown that banana peels possess antioxidant and antiinflammatory properties. This study was performed to evaluate the wound healing activity of banan peels extract (BPE) in rabbit. For inducing full-thickness wound in rabbits, the excisional wound model was used. The animals were randomly divided into six experimental groups. Negative control, standard and vehicle control groups and treatment groups. All the treatment were apllied topically twice daily. Healing was assessed by wound contraction and re-epithelialization rate and the tensile strength of wound tissue sample . Histopathological examination was also done.The BPE promoted wound healing activity in full-thickness wound model. High rate of wound contraction ($P<0.05$), significant decrease in epthelialization period ($P<0.05$) and high skin breaking strength ($P<0.01$) were obsereved in animals treated with BPE. Histopathological sutudies also showed the wound healing activity of BPE.The results of this study indicated that hydroalcoholic extract of banana peels has a strong potetial for wound healing and it can be used for different types of wounds in human being too.

Key words: Wound healing, Banana peel, *Musa acuminata*, Rabbit

Introduction

A wound is defined as physical injury that result in an opening or break of the skin that causes disturbance in normal skin anatomy and function [1] Wound healing is body's natural reaction to tissue injury involving a series of cellular events that generates resurfacing, reconstitution, and restoration of the tensile strength of injured skin. Normal wound healing is a complex process consists of four highly integrated and overlapping phases: hemostasis, inflammation, proliferation, and tissue remodeling or resolution [2]. Wound care is constantly evolving with the advances in medicine but wounds are still a significant health problem worldwide, often having severe complications. In recent years wound care professional revisited the ancients healing methods by using traditional medicine in wound management. Traditional medicines which are more effective, nontoxic and cost-effective are gaining popularity throughout the world. The medicinal and biological effects of plants has urged scientists to examine these plants with a view to determine potential wound healing properties [3]. Musa acuminata (Musaceae) is one of the most widely distributed and consumed fruit in the world. Considering the nutritional aspects, it is one of the world's leading food crops with a great source of minerals, vitamins, carbohydrates, flavonoids and phenolic compounds [4,5]. All parts of banana plants have been used in traditional medicine to treat a variety of diseases [6]. The fruit has been used in diarrhoea, dysentery, intestinal lesions in ulcerative colitis, diabetes and as a dressing for burns [7], Banana leaves (ashes) are used in eczema [8], Flowers are used in dysentery and menorrhagia. Stem juice of fruited plant is used for treating diarrhoea, dysentery, cholera, otalgia and haemoptysis [7].

The peels of a variety of fruits have gained attention as a natural source of antioxidants and phytochemical content which are rich in compounds with free radical scavenging activity compounds [9]. The banana fruit, peel and trunk from various species and sampling areas have been reported to be rich in essential minerals, mainly containing high concentrations of potassium [10-12]. Banana peel is an underutilized source of phenolic compounds is considered as a good source of antioxidants for foods and functional foods against cancer and heart disease [13]. The research on banana (Musa acuminata) peel extract indicated that banana peel is potential source of bioactive compounds like flavonoids and polyphenols with wide range of medicinal properties in particular the high free radical scavenging activity [14]. Antifungal and antibiotic principles are found in the peel and pulp of fully ripe bananas [15]. The present study has been aimed to evaluate the wound healing activity of hydroacoholic extract of banana (Musa acuminata) peels (BPE) on full thickness wounds in rabbit.

Methods

Plant material

Banana fruits were purchased from local market, Ahvaz, Iran. The plant was identified as Musa acuminata in Department of Pharmacognosy, School of Pharmacy, Jundishapur University of Medical Sciences, Ahvaz, Iran. A voucher specimen of the sample has been deposited in the herbarium of the Department. The peels were separated from fruit and washed. The peel was shade dried for two weeks. Dried peels were ground to make a coarse powder. The powdered peels (500 g) were soaked in ethanol 70% for 72 hours in laboratory temperature. The filtrated extract was evaporated under vacuum below 45◦C to give a final yield of 25·5g. For use in experiment, the dried powder was mixed with eucerin for preparing the 3%, 5% and 10% creams.

Animals

Iranian rabbits of either sexes weighing 1300–1800g were used for the study. They were individually housed in stainless steel cages at a 12-hour cycle of light and dark, room temperature was kept at 24±2◦C and humidity maintained at 50%. Standard food, vegetables and water was provided ad libitum. The rabbits were used after acclimatation to the laboratory environment for a 7day period. Animal procedures were in accordance to the guidelines for animal care prepared by the Animal Ethical Committee of Ahvaz Jundishapur University of Medical Sciences (approval code no. 04/2015/CPCSEA/AJUMS).

Wounding procedure

Full thickness wounds were made in the skin of the animals according to the model of Cross et al. [16] Hairs of lower back and left flank of the animals were fully shaved and sterilized with 70% ethanol, the desired area was locally anaesthetized with the subcutaneous injection of lidocaine (2%). A metal template measuring 20x20 mm was placed on the stretched skin and an outline of the template was traced on the skin using a fine-tipped pen. The wound was made by excising the skin, within the border of the template to the level of loose subcutaneous tissue (facia), using a size 15 scalpel

blades and a forceps. A progressive decrease in the wound area was monitored every day. The surface area of healing wound was measured by tracing the boundary of still open wound on transparent paper and calculation of area was done by using a scale graph paper. The percent wound contraction was calculated using the following formula:

$$\%\text{wound contraction} = \frac{\text{Initial wound size} - \text{Specific day wound size}}{\text{Initial wound size}} \times 100$$

The animals were divided into six groups (6 animals per group) as standard, negative control, vehicle control and three groups for testing wound healing activity of banana peels extract. The standard group was treated with phenytoin1% cream, the negative control group received no treatment, vehicle control group was treated with eucerine and test groups were treated with 3%, 5% and 10 % creams of BPE, respectively. All the treatments were applied topically twice a day from the day 0, 4 h after the creation of the wounds till the complete healing.

Epithelialization period

It was evaluated by noting the number of days required for the scar to fall off from the wound surface exclusive of leaving a raw wound behind [17].

Histopathological studies

Histopathological studies were performed on day7th of treatment. Skin specimens from treated and untreated rabbits were collected and fixed in 10% buffered formalin and after the usual processing, 5-µm thick sections were cut and stained with haemtoxylin and eosin. Sections were qualitatively assessed under light microscope.

Tensile strength

The tensile strength of a wound represents the degree of tissue integrity. The tensile strength increment indicates better wound healing stimulation by the applied drug. At the end of healing period, a strip of repaired tissue measuring 20 × 5 mm was isolated and the tensile strength was measured with tensiometer. Tensile strength was calculated using the following formula [18]:

$$\text{Tensile Strength} = \frac{\text{Breaking strength(g)}}{\text{Cross} - \text{sectional area of skin } (\text{mm}^2)}$$

Statistical analysis

Data were expressed as mean ± SE and statistical significance between experimental and control values were analyzed by one-way analysis of variance (ANOVA) and followed by Dunnett,s test to identify differences between groups. Values of $P<0.05$ were considered as statistically significant.

Results

Wound contraction and epithelialization

There was no significant difference in wound contraction between the non-treatment group and eucerin-treated group throughout the study period. The 10% cream of BPE improved the wound healing at all times beginning on the 3rd day with statistical significance ($P < 0.05$) achieved from 7th day as compared with the eucerin-treated and no treatment groups. Period of complete healing in non-treated and eucerin-treated groups were equal over 21 days. Healing in the group treated with phenytoin cream1% was completed within 16 days. Healing in animals treated with creams containing 3%, 5%, and10% of BPE occurred after 19, 18 and 15 days, respectively. A statistical significant decrease in epithelialization time in BPE 10% ($P<0.01$) and phenytoin treated groups ($P<0.05$) was observed as compared with eucerin-treated and no-treatment groups (Figure 1). The 10% cream of BPE healed the wounds in a short duration compare to the other experimental groups.

Histopathological studies

The wound healing activity of the extract was confirmed by histological investigation. On the 7th day of treatment with 5% and 10% cream of BPE in most samples, granulation tissue and reconstruction of epidermis was initiated whereas in no treatment, eucerin or 3% cream treated groups no sign of repair was evident and still necrosis was observed (Fig.2).

Tensile strength

The BPE (5% and 10%) significantly ($P<0.01$) increased the tensile strength of wound samples compare to the control groups (Table. 1)

Discussion

Wound represents a major health problem, both in terms of morbidity and mortality. Treatment of injury and physical damage are requires special care and costs abundant. A therapeutic agent selected for wound healing should improve the healing process with minimum side effects. Plants products are potential agents for wound healing and largely preferred because of their widespread availability,

cost effectiveness, non- toxicity and their effectiveness as crude preparations [3]. Previous studies have shown some species of banana such as Musa sapientum has a potential for wound healing [19,20] but to the best of our knowledge no study have been done to evaluate the wound healing effects of Musa acuminata. In the present study we investigated the wound healing activity of Musa acuminata and our results showed that 10% cream made of hydroalcoholic extract of Musa acuminata peels accelerate the wound healing process in full thickness wounds. Healing was assessed by wound contraction and epithelialization rate and the tensile strength of wound tissue sample. Histopathological studies was also performed.

Experimental and clinical evidence suggest that chronic wound undergoes substantial oxidative stress by neutrophils-derived oxidants and myeloperoxidase inflammation [21] The over production of reactive oxigen species (ROS) result in oxidative stress with detrimental cytotoxic effects causing delayed wound healing, therefore elimination of ROS could be an important strategy to improve healing of chronic wounds [22]. Previous studies have shown that Musa acuminata is a potential source of bioactive compounds like flavonoids and polyphenols with wide range of medicinal properties in particular the high free radical scavenging activity [13,23] and this may explain in part the mechanism of healing effects of banana peel extract on skin wounds. The antibacterial, immunostimulatory and physiological regulatory effects of banana peel extract were reported by Rattanavichai et al. [24]. The healing property of Banana Peel Extract may be due to the fact that it offers antibacterial activity, stimulate the immune system and regulate the physiological process such as healing process.

Conclusion

The results of our study showed that the Banana Peel Extract improve the wound healing process in the experimental model. This finding could justify the inclusion of this plant in the management of wound healing.

Acknowledgements

We would like to express our special thanks to the Deputy of Research and Technology Development, Ahvaz Jundishapur University of Medical Sciences. This study has been adapted from an Pharm D thesis at Ahvaz Jundishapur University of Medical Sciences ((Grant No DRT.234) .

References

1. Strodtberck F. physiology of wound healing. newborn infan nurs rew. 2001;1:43-52.
2. Gosain A, DiPietro LA. Aging and Wound Healing. World J Surg. 2004;28(3):321-6.
3. Thakur R, Jain N, Pathak R, Sandhu SS. Practices in Wound Healing Studies of Plants. Evidence-based Complementary and Alternative Medicine : eCAM. 2011;2011:1-17.
4. Mohammad ZI, Saleha. A. Musa paradisiaca L. and Musa sapientum L.: A Phytochemical and Pharmacological Review. Appl Pharmaceut Sci. 2011:14-20.
5. Guyle`ne Aurorea B, P., & Louis, F. Bananas, raw materials for making processed food products. Tren Food Sci, Technol. 2009(20):78-91.
6. Amit SS. Limonoids: Overview of significant bioactive triterpenes distributed in plants kingdom. Biol Pharm Bull. 2006;29(2):191-201.
7. Ghani A. Medicinal Plants of Bangladesh: Chemical Constituents and Uses. 2nd, editor. Dhaka, Bangladesh: The Asiatic Society of Bangladesh,; 2003.
8. Okoli RI, Aigbe O., Ohaju-Obodo J.O., Mensah J.K. Medicinal Herbs Used for Managing Some Common Ailments among Esan People of Edo State, Nigeria. . Pakistan J Nutr. 2007;6(5):490-6.
9. Komolafe JOAaEA. Antioxidative Potentials of Banana and Plantain Peel Extracts on Crude Palm Oil. Ethnobot Leaflets. 2010;14(559-569).
10. Twyford IT, Walmsley D. The mineral composition of the Robusta banana plant. Plant and Soil. 1973;39(2):227-43.
11. Cordeiro N, Belgacem MN, Torres IC, Moura JCVP. Chemical composition and pulping of banana pseudo-stems. Indus Crop Prod. 2004;19(2):147-54.
12. Haslinda WH, Cheng LH, Chong LC, Noor Aziah AA. Chemical composition and physicochemical properties of green banana (Musa acuminata x balbisiana Colla cv. Awak) flour. International journal of food sciences and nutrition. 2009;60 Suppl 4:232-9.
13. Someya S, Yoshiki Y, Okubo K. Antioxidant compounds from bananas (Musa Cavendish). Food Chem. 2002;79(3):351-4.
14. Singhal S, Prakash, R. . Antioxidant Activity, Total Flavonoid and Total Phenolic Content of Musa acuminate Peel Extracts. Glob Jf Pharmacol,. 2013;7:118-22.
15. Brook AA. Ethanol production potential of local yeast strains isolated from ripe banana peels. Afri J Biotechnol. 2008;7(20):254-7.
16. Cross SE, Naylor, I., Coleman, R.T. and Teo, T.C. An experimental model to investigate the dynamics of wound contraction. Br J Plast Surg. 1995;48:189-97.
17. Rashed AN, Afifi FU, Disi AM. Simple evaluation of the wound healing activity of a crude extract of Portulaca oleracea L. (growing in Jordan) in Mus musculus JVI-1. J Ethnopharmacol 2003;88(2-3):131-6.
18. Brown G CLJ, White M, Mitchel R, Pietsch J, Nordquist R. et al. Acceleration of tensile strength of incisions treated with EGF and TGF-β. Annua Surg 1988;208:788.
19. Atzingen DANCV, Gragnani A, Veiga DF, Abla LEF, Cardoso LLF, Ricardo T, et al. Unripe Musa sapientum peel in the healing of surgical wounds in rats. Acta Cir Bras. 2013;28:33-8.
20. Agarwal PK, Singh A, Gaurav K, Goel S, Khanna HD, Goel RK. Evaluation of wound healing activity of extracts of plantain banana (Musa sapientum var. paradisiaca) in rats. Indian journal of experimental biology. 2009;47(1):32-40.
21. Song HS, Park TW, Sohn UD, Shin YK, Choi BC, Kim CJ, et al. The effect of caffeic acid on wound healing in skin-incised mice. Korean J Physiol Pharmacol. 2008;12(6):343-7.

22. Mikhal'chik E, Anurov M, Titkova S, Miroshnikova E, Lukasheva E, Deeva I, et al. Activity of antioxidant enzymes in the skin during surgical wounds. Bull experiml biol, med 2006;142(6):667-9.
23. Singhal S, Prakash, P. valuation of Antioxidant Activity of Banana Peels (Musa acuminata) Extracts Using Different Extraction Methods. . Chem Sci Transactions. 2014;4:158-60.
24. Rattanavichai W, Cheng W. Effects of hot-water extract of banana (Musa acuminata) fruit's peel on the antibacterial

Table 1. Comparison of tensile strength of tissue sample in different groups

Groups	Tensile strength (g/cm^2)
No-treatment	995± 33
Eucerin	998±32
BPE3%	1170±36
BPE5%	1311±45**
BPE10%	1323±41**
Phenytoin1%	1289 ± 35*

*P< 0.05, **P < 0·001 (significant differences from no-treatment or eucerin treated groups)

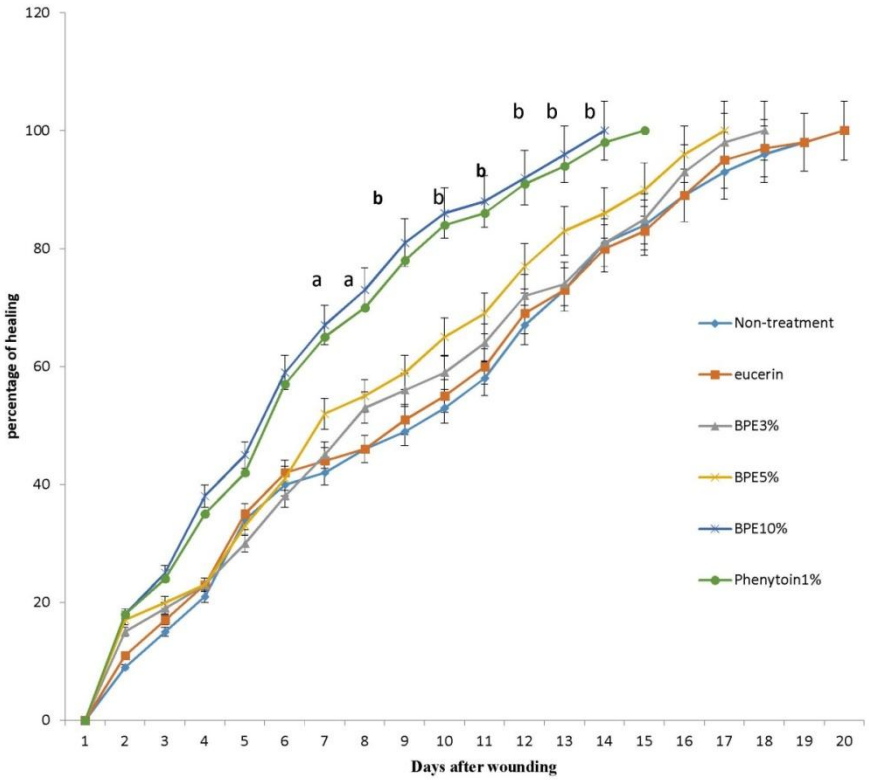

Figure 1. Comparison of the wound healing in experimental groups. Data are expressed as mean ± SE. Values significantly different from eucerin-treated or no-treatment are indicated as (aP<0.05, b P< 0·01). BPE: Banana peel extract

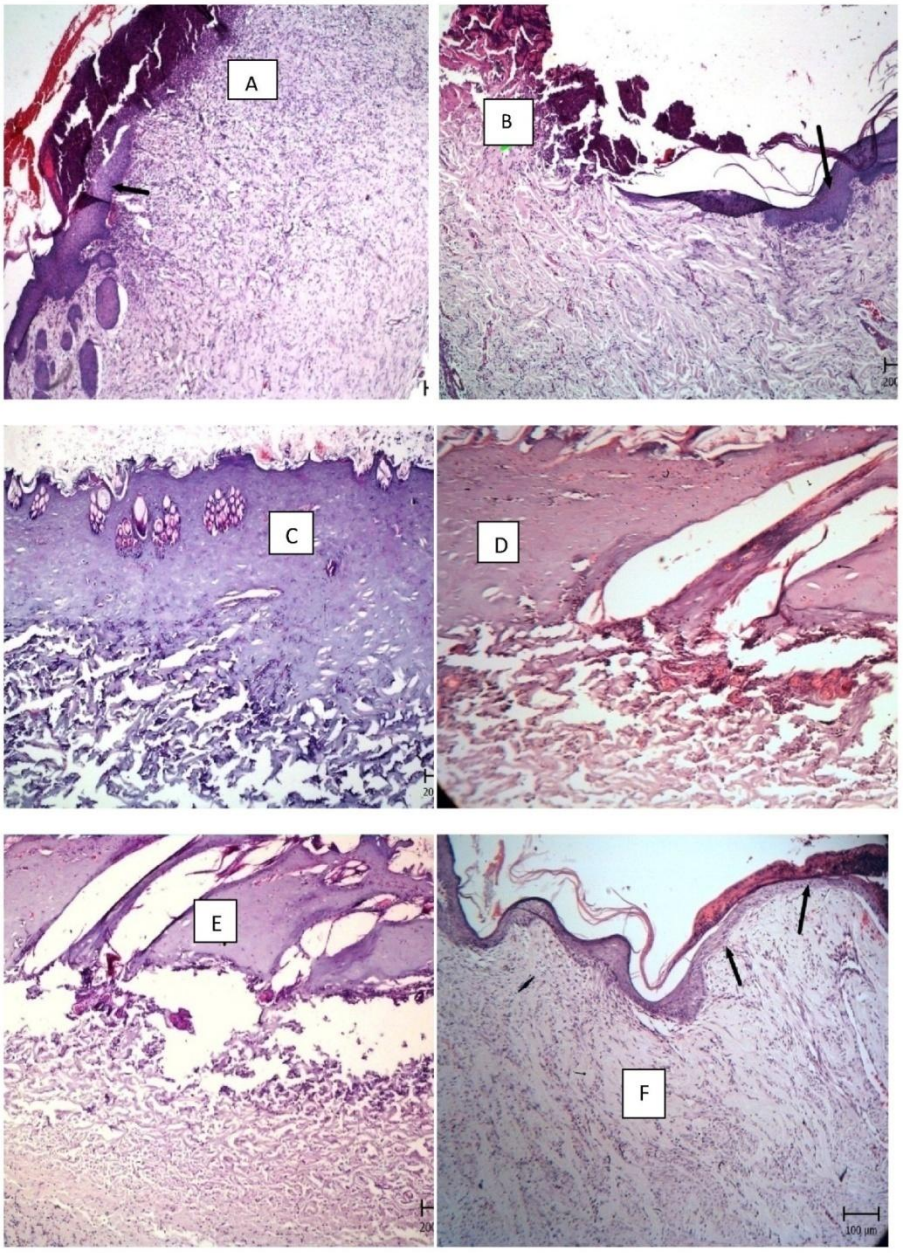

Figure .2 Histopathology of skin at day 7, stained with H&E (100x). (A) Skin of BPE10% treated rabbits. Large amount of granulation tissue and extensive fibrosis can be seen. (B) Skin of BPE5% treated rabbits. Large amount of granulation tissue and small numbers of inflammatory cells can be seen. (C) BPE 3% treated, (D) Eucerin treated or (E) no treatment rabbits. Necrosis and inflammation are still evident. (F) Skin of phenytion1% treated rabbit. Evidence of epithelialization (arrows) with dermal proliferation of fibroblsats.