

Short communication

The Wound Healing Effect of *Plantago Major* Leaf Extract in a Rat Model: An Experimental Confirmation of a Traditional Belief in Persian Medicine

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Abstract

Background and Aim: *Plantago major* leaf has been traditionally used in Iran and many other countries for wound healing. This study presents a brief report about the depiction of the effects of *Plantago major* on wound healing in the major texts of Persian medicine. Moreover, the effect of *Plantago major*'s leaf extract on wound healing duration has been experimentally assessed in male rats.

Materials and Methods: In experimental studies, the methanolic extract of *Plantago major*'s leaf was used as an ointment. To make a wound model, a circular ulcer was made on the back of animals. Adult male Wistar rats were divided into two groups: animals in the control group were treated once a day only with the ointment's eucerin base, and the rats in *Plantago major*'s group were treated with the ointment containing the plant extract. Ulcerous areas were measured on days 0 and 14. The durations of complete wound healing processes were determined too.

Results: The difference between the mean duration of wound healing was statistically significant using independent samples t-test (20.7 ± 0.4 days in control vs 19.1 ± 0.4 in plant extract group, $p=0.022$). Furthermore, there was a significant difference in the mean wound surface area on the fourteenth day ($p=0.014$) despite the fact that there were no significant differences in day 0 ($p=0.69$).

Conclusion: There is a long history of using *Plantago major*'s leaf for wound healing in Persian medicine text books. It was determined, in the experimental studies conducted on rats, that *P. major*'s leaf extract could accelerate wound healing process. This capability justifies its application not only in Persian medicine but also in some other traditional medicines.

Keywords: *Plantago major* L, wound healing, traditional Persian medicine, Iran

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Introduction

Ulcer is a disruption of the continuous building of body which is the result of physical or chemical injury (1). Wound healing is a complicated, multi – step process that can be divided into four major phases: inflammation, proliferation, and scar formation/remodeling (2). Traditional medicine has demonstrated the therapeutic efficacy of some medicinal herbs in wound healing. The *Plantago major* L. is widely known as a perennial plant which is classified as a member of the Plantaginaceae family. The height is about 15 cm but varies depending on the growth habitats. The leaves are glabrous and have an dentate margin (3).

This old herb has a variety of uses in traditional medicine of many countries. It has been used since a long time ago to cure the common cold, respiratory diseases, infectious diseases, problems related to digestive organs, hepatitis and skin disease. It has also been used as an ointment in the treatment of pimple, ulcer and stye (3, 4). Moreover, in many countries, including Mexico, Argentina, Chile and Venezuela many reports demonstrating the traditional use of *P.major* against cancer have been presented (5, 6).

New studies have shown that this herb can significantly decrease the number of coughs. It has anti-asthma and anti-dyspnea effects. The major flavonoid of *P.major* (Luteolin-7-O- β -glucoside) is used as a potent anti-cancer with inhibitory effects on breast adenocarcinoma. Most parts of this perennial herb are used as medicine for digestive and circulatory disorders, preventing tumor formation, skin diseases and healing wounds. *Plantago*'s laxative application, its usage for hypercholesterolemia and its ability in decreasing blood sugar have been confirmed by World Health Organization (4).

Plantago major L., which is called "Lesan al-Hamal" or "Barhang", has been used as a wound healing remedy in Persian Medicine. In a brief exploration in some of the major Persian medicine text books, it

was determined that Rhazes declared in his prominent book, Al- Havi, that *P.major* leaf could be used to treat chronic wounds and mucosal ulcers (7). Avicenna declared, in his outstanding medical book "Al Qanun Fi al tibb", that the leaves of *P.major* could recover deep and old wounds (8). Furthermore, Hakim Momen Tonekaboni and Aghili Khorasani, two of the most prominent physicians of Persian medicine who lived in the seventeenth and nineteenth centuries respectively, proposed this plant for healing the wounds (9, 10) (Fig. 1).

Despite the widespread reports concerning the use of this plant's leaves for wound healing in traditional medicine, only a few experimental studies have indicated that *P.major* leaf extract has useful effects on wound healing (4). The aim of the present study was primarily the evaluation of the effect of wild Iranian *P.major* leaf extract on the duration of wound healing in a rat model.

Materials and Methods

Animals

Sixteen male rats weighing between 200 g to 250 g were used in this study. The animals were randomly divided into two groups, 8 rats per group. They were housed in separate cages at a controlled temperature under a 12-h light/ dark cycle and received a standard diet. All animal experiments were approved by the Animal Care and Use Committee at the Lorestan University of Medical Sciences, Khorramabad, Iran.

Extraction

Plantago major L leaves were collected from a village (Einabad) at the vicinity of Saveh, a city in Markazi Province of Iran. Plant collection was done in spring, and leaves were dried in shadow. 100.0 g pulverized *P. major* leaves were extracted by stirrer apparatus (IKA, Germany) for 48 h with 1L methanol. The insoluble material was separated by filtration (Whatman No. 3 paper), and dried by a rotary evaporator (Heidolph, Germany). Extraction yield was 37.33%.

Wound Model

For surgical proceedings, the animals were weighed

and then sedated with a subcutaneous injection of 5 mg/kg diazepam and then anesthetized after 10 minutes with subcutaneous ketamine injection (25 mg/kg). When anesthesia and the shaving the animal's back area were accomplished, a circular ulcer (diameter=2 cm) was made on the back of each animal by a pair of surgical scissors. The rats in the treatment group received ointment containing methanolic *P. major* leaf extract in eucerin base, once a day. The rats in the control group received only the eucerin base of the ointment without the *plantago major* extract.

On the days 0 and 14, the ulcer's area was measured by graph paper after drawing of the wound margins on a transparent paper. Furthermore, the health day was documented by the meaning of complete ulcer's closure, hence, the total duration of wound healing process was determined.

Statistical Analysis

The data of healing time duration and wound surface area were expressed as mean±SD. To compare the two groups, independent samples t test was used. IBM SPSS Statistics version 19 was used for statistical analysis. Graphs were drawn by GraphPad Prism version 5. $P \leq 0.05$ was considered as significant.

Results and Discussion

There was no significant difference in the wound surface area in day 0, i.e., at the beginning of the study ($p=0.69$). No signs of abscess formation in the first phase (zero days) or hypertrophic scars in the last phase (14days) were observed. The mean time of wound healing in the treatment group was 19.1 ± 0.4 compared to 20.7 ± 0.4 days in the control group. The difference was statistically significant ($p= 0.022$). (Figure 2). Moreover, the median time of wound healing in the treatment group was 19 days (range: 17-21 days) and the median time of wound healing in the control group was 21 days (range: 19-22 days). Hence, the extract of the leaf of *Plantago L major* could accelerate wound healing for approximately two days in this rat model. Wound surface area on the fourteenth day was also significantly lower in the group that used *P. major* extract compared to the control group ($p=0.014$, Figure 3).

It was already noted in present study that the *P.*

major has been used in Persian medicine for wound healing. Our pilot data on wound closure time and wound surface area on the fourteenth day in a rat model confirmed this traditional use of *P. major* leaves. Wounds are considered as major clinical problems which can reduce the survival rate and quality of life in injured people. Successful wound healing is a major healthcare and biomedical challenge in the present century. There is no quite successful therapy for wound healing. It has high health care costs, and attracts the attention of medical communities (11).

Studies conducted on medicinal plants have confirmed that herbal drugs are more cost-effective and exhibit fewer side effects in comparison with chemical agents (12, 13). Furthermore, the clinical applications of some herbal medicines for diabetic foot infections and decubitus ulcers have resulted in successful courses of treatment (14). The purpose of the present study was to determine the efficiency of *P. major* ointment on shortening the wound healing duration in a rat model. *Plantago major* has a number of unique properties that make it suitable to be widely used in traditional medicine. This perennial herb has been used for centuries in the treatment of cold, hepatitis, skin diseases, infectious diseases, and problem related to digestive organs, respiratory organs, reproduction, circulation and fever (3). Moreover, its topical ointment is used against boils and ulcers (15).

Greek physicians prescribed the leaves of this plant to treat dog bites in the first century (3). Vikings used *P. major* leaves for wound healing in the twelfth and thirteenth centuries. Ibn Ei Beithar, an Islamic author, described *P. major* too. Hemrik Harpestreng from Denmark wrote that *P. major* could cure everything. In Scandinavia, the *P. major* is primarily known for its wound healing properties. This plant has been used for wound healing in traditional medicine of Turkey, Brazil, Philippines, Denmark, Norway and Sweden (3).

Traditional Iranian medicine resources contain a wide range of special natural products and medicinal protocols for the management of complicated diseases that modern medicine has been unable to effectively treat. In traditional Iranian medicine, *Plantago major*'s name is Barhang or Lesan-ol-haml and it has been used against hemorrhage, diarrhea, peptic ulcer, IBD,

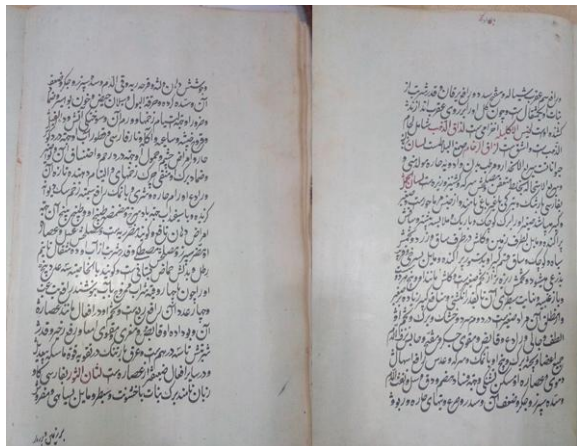


Figure 1. The pages on "Lisan al-Hamal" (*Plantago major* L.) explaining its different indications, including healing the wounds in the book of "Tohfeh Hakim Momen" (The gift of Hakim Momen): an original manuscript in Persian medicine (Written in 1080 AH, AD), PP: 132 &133, kept in Library of the School of Persian Medicine, Babol University of Medical Sciences, Babol, Iran (No. 2084).

wound and injury (16). Both the ethanol and water extracts of *P. major* Leaves and seeds could gastric ulcer induced by aspirin administration in a rat model, and the ethanolic extract of the leaves had better gastroprotective effects than other extracts (17). The anti-peptic ulcer activity of the *P. major* leaf extract also has been confirmed as a dose dependent effect in various rodent models of peptic ulcer (18). Yesilda *et al.* used the powdered dried extracts of *P. major* leaves with honey as a pretreatment in a rat water immersion-stress ulcer model. In rats which received combined methanol and the water extract of *P. major*, ulcer formation was inhibited relative to the control group (19). More recently, Zubair *et al.* reported that *P. major* leaf extract could stimulate skin wound-healing activities in an ex-vivo porcine wound model (20). Wound healing is a complicated process that involves the following stages: hemostasis, inflammation, differentiation, proliferation, and migration of mesenchymal cells to the wound site, angiogenesis, re-epithelialization (re-growth of epithelial tissue over the damaged surface), and collagen's synthesis, cross-linking and alignment to provide strength of the healing tissue (21). The capability of anti-oxidants in wound healing progression has been confirmed (22, 23). Moreover, anti-inflammatory agents have a key role in wound healing and preventing wound exacerbation (24).

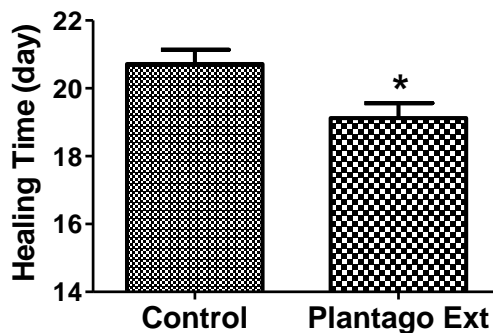


Figure 2. Wound healing duration in the control and treated groups with *Plantago* leaf extract (*Plantago* Ext). * Significantly different from control group.

Furthermore, anti-microbial agents are efficient in the management of microbial infections which may concomitantly occur in chronic and severe wounds and may be involved in better wound healing (13). Antioxidant and anti-bacterial activities of *P. major* leaves, which might be effective in wound healing, have been indicated (12, 21). *P. major* contains antioxidant compounds such as plantagin, baicalein and hispidulin. Such compounds might be somewhat responsible for cell protection against destruction by inflammatory mediators (25, 26). The biotic mechanism of wound healing is remarkably similar in almost all kinds of tissues regardless of the alterations in the type of injury and the tissue involved. As several studies have indicated, the wound healing process starts with inflammatory responses followed by the generation of new tissue and granulation, recruitment, and growth of endothelial cells for

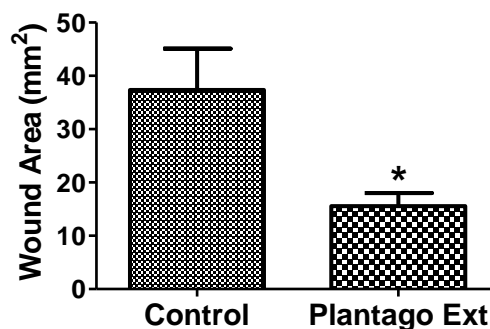


Figure 3. Wound surface area on the fourteenth day in the control and treated groups with *Plantago* leaf extract (*Plantago* Ext). * Significantly different from control group.

angiogenesis (14).

Cell migration and proliferation play significant roles in wound healing process. Different bioactive substances, such as polyphenols and polysaccharides can stimulate cell proliferation and migration (27), and may contribute to ameliorated wound healing process after being localized using *P. major's* leaf extract. Moreover, it has been shown that neo-epithelium and skin appendages formation are more efficient in animals which received *P. major* compared to the control group (28).

Conclusion

The topical administration of *Plantago major's* leaf extract accelerated the wound healing process in a rat model. This fact, justifies the application of this plant in Persian medicine and some other traditional medicines. Further researches are required to determine the underlying mechanisms.

Acknowledgment

None.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Johnston DE. Wound healing in skin, plastic and reconstructive surgery. *Vet. Clinic. North. Am.* 1990; 20(1):1-45.
2. Schwartz, S. *Principles of surgery*. 4th ed. Newyork :Mc.grawhill company. 1984;289-304.
3. BeritSamuelsen A. The traditional uses, chemical constituents and biological Activities of *Plantago major* L. A review, *Journal of Ethnopharmacology*. 2000;71:1–21.
4. Kazhal Haddadian et al. A review of *plantago* plant. *Indian journal of traditional knowledge*. 2014;13(4):681-5.
5. Galvez M. cytotoxic effect of *plantago* spp. on cancerallins. *J Ethnopharmacol*. 2003;88:125-30.
6. srivastava et al. plant based anticancer molecules: A chemical and biological profile of some important leads. *Bioorganic med chem*. 2005;13:5892-590.
7. Razi M. Al-Havi (The Large Comprehensive), Beirut: Dare Ehia Attorath Al Arabi; 2001.
8. Ibn-e-Sina AH. *Al-qanun fit-tib* [The canon of medicine].

Beirut, Lebanon: Alaalami Beirut lib Press; 2005.

9. Aghili Khorasani MH. In: Makhzan-ol-Adviyah [Storehouse of Medicaments]. Shams Ardakani MR, Rahimi R, Farjadmand F, editors. Tehran: Tehran University of Medical Sciences; 2009.

10. Momen T. Tohfatal-Momenin (Tohfehe-Hakim Momen). Traditional medicine of Research center of Shahid Beheshti University of Medical Sciences Nashre Shar constitute, Tehran, Iran. 2008.

11. Rizzi SC, Upton Z, Bott K, Dargaville TR. Recent advances in dermal wound healing: biomedical 20. device approaches. *Expert Rev Med Devic*. 2014;7:143-54.

12. Kumar B, Vijayakumar M, Govindarajan R, Pushpangadan P. Ethnopharmacological approaches to wound healing-- exploring medicinal plants of India. *J Ethnopharmacol*. 2007;114(2):103-13.

13. Rhoads DD, Cox SB, Rees EJ, Sun Y, Wolcott RD. Clinical identification of bacteria in human chronic wound infections: culturing vs. 16S ribosomal DNA sequencing. *BMC Infect Dis*. 2012;12:321.

14. Gurtner GC, Werner S, Barrandon Y, Longaker MT. Wound repair and regeneration. *Nature*. 2008;453(7193):314.

15. kuhn M A et al. herbal therapy and supplements: a scientific and traditional approach, (lippincot Philadelphia). 2000;262-267.

16. Mohammad Hosein Farzaei et al. A Comprehensive Review of Plants and Their Active Constituents with Wound Healing Activity in Traditional Iranian Medicine. *Wounds*. 2014;26(7):197-206.

17. Kobeasy M, Abdel-Fatah OM, El-Salam SA. Gastroprotective effect of *plantago major* L. against gastric injury induced by aspirin in rats. *Journal of Chemica Acta*. 2013;2(2):86-91.

18. Melese E, Asres K, Asad M, Engidawork E. Evaluation of the antipeptic ulcer activity of the leaf extract of *Plantago lanceolata* L. in rodents. *Phytotherapy research*. 2011;25(8):1174-80.

19. Yesilada, E., Sezik, E., Fujita, T., Tanaka, S., Tabata, M., 1993. Screening of some Turkish medicinal plants for their antiulcerogenic activities. *Phytotherapy Research* 7, 263–265.

20. Zubair M, Nybom H, Lindholm C, Brandner JM, Rumpunen K. Promotion of wound healing by *Plantago major* L. leaf extracts—ex-vivo experiments confirm experiences from traditional medicine. *Natural product research*. 2016;30(5):622-4.

21. Gosain A, DiPietro LA. Aging and wound healing. *World J Surg*. 2004;28:321-6.

22. Martin A. The use of antioxidants in healing. *Dermatol Surg*. 1996;22(2):156-60.

23. Süntar I, Akkol EK, Nahar L, Sarker SD. Wound healing and antioxidant properties: do they coexist in plants? *Free Radicals Antioxidants*. 2012;2(2):1-7.

24. Sen CK, Roy S. Redox signals in wound healing. *Biochim Biophys Acta*. 2008;1780(11):1348-61.

25. Muhammad Bahrain Adoma et al. Chemical constituents and medical benefits of *Plantago major*. *Biomedicine & Pharmacotherapy*. 2017;96:348-60.

26. Yokozawa T. Antioxidative activity of flavones & flavonols in vitro. *Phytother. Res*. 1997; 11 (6) 446–449.

27. Zubair M, et al., Effects of *Plantago major* L. leaf extracts on oral epithelial cells in a scratch assay, *J. Ethnopharmacol*. 2012;141(3):825–30.

28. Thomé RG, dos Santos HB, dos Santos FV, et al. Evaluation of healing wound and genotoxicity potentials from extracts hydroalcoholic of *Plantago major* and *Siparuna guianensis*. *Exp Biol Med (Maywood)*. 2012;237(12):1379-86.