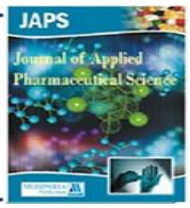
Journal of Applied Pharmaceutical Science

Available online at www.japsonline.com



ISSN: 2231-3354
Received on: 27-08-2011
Revised on: 02-09-2011
Accepted on: 05-09-2011

Wound healing activity of ethanolic extract of *Plantago Ovata* (Ispaghula) seeds

Sujata Singh, Rashmi Singh, Neeraj Kumar, Rajnish Kumar

Sujata Singh, Rashmi Singh
Department of Pharmacology, Shri Ram Murti Smarak Institute of Medical Sciences, Bareilly, UP, India

Neeraj Kumar
Department of Pharmacy, Shri Ram Murti Smarak College of Engineering And Technology, Bareilly, UP, India

Rajnish Kumar
Department of Pharmacy, Shri Ram Murti Smarak College Of Engineering And Technology, Bareilly, UP, India

For Correspondence
Dr. Sujata Singh, MD,
Assistant Professor, Department Of Pharmacology, Shri Ram Murti Smarak Institute Of Medical Sciences, Bareilly, UP, 243202
Phone No.-9359169974

ABSTRACT

Present work was done to explore the wound healing activity of ethanolic extract of Plantago ovata seeds in albino wistar rats. The extract was tested for wound healing activity by excision and incison wound model. The extract was used as ointment (10% w/w) in petroleum jelly base. The extract showed significant response ($p<0.01$) in both the wound types tested when compared with the control group. Aloe vera ointment (10%w/w) was used as standard drug and the activity of the extract was in close proximity to standard. On the basis of the results it can be said that the extract of Plantago ovata seeds possess wound healing activity.

Key words: Wound healing, excision, incision, Plantago, Ispaghula

INTRODUCTION

Wounds are the visible results of cell death or damage, which can be classified on the basis of site, size, depth and causation like surgery, accident or circulatory failure (Charde et al, 2010). The objective in wound management is to heal the wound in the shortest time possible, with minimal pain, discomfort, and scarring to the patient. In addition to this a flexible and fine scar with high tensile strength is also desired. Hence understanding the healing process is critical to successful management of wound. Wound healing is the process of repair that follows injury to the skin and other soft tissues. This process consists of integrated cellular and biochemical events leading to the re-establishment of structures and functional integrity and regaining strength of the injured tissue (Gupta and Jain, 2010). A number of drugs ranging from simple non-expensive analgesics to complex and expensive chemotherapeutic agents administered in the management of wound affect healing either positively or negatively (Prasad and Rao, 1995). Aspirin, indomethacin, cytotoxic agents and immunosuppressants have been proved experimentally to affect healing negatively (Lee, 1968, Rao et al, 1991 Raju and Kulkarni, 1986, Holla et al, 1988). Medicinal herbs are an indispensible part of traditional medicine and these have been the basis of treatment and cure for various diseases and physiological conditions in traditional methods practiced such as Ayurveda, Unani and Siddha. India has a rich flora that is widely distributed throughout the country.

One of the Indian plants Plantago ovata (P. ovata) belonging to family Plantaginaceae, commonly called 'Ispaghula Husk', is largely produced in Punjab, Haryana, Gujarat, Rajasthan and Madhya Pradesh. The plant grows 65 to 90 cms height, about 50 to 90 bunches comes out from the plant like grapes bunches (agricultureinformation.com). The seed contains mucilage in the epidermis of testa, which on hydrolysis yields D-xylose, L-arabinose, aldobiuronic acid, aucubin glycoside, sugars, sterols and protein. The seeds have been found to be effective in

chronic constipation , Ulcer, Piles, both type of diabetes and has been also shown to possess demulscent and anti-inflammatory activity (Evans, 2002, Rodriquez et al, 2003, Hannan et al, 2006). Although many indigenous tribes around the world have long been suspected that this ubiquitous, annual, herbaceous plant might have medicinal wound healing properties, it has not really got the attention of orthodox medical practitioners as a potential source of a healing agent which may prove to be useful in the treatment of wounds. Therefore the present study was planned to explore the wound healing activity of P. ovata seeds.

MATERIALS AND METHODS

The work was conducted in the department of pharmacy, Shri Ram Murti Smarak College of Engg And Technology Bareilly, UP (India) in June 2011.

Plant material and preparation of herbal extract

P. ovata seeds were purchased from specialized herbal medicine shop (GM Pharmacy, Bareilly, UP (India)) and authenticated by its morphological characteristics (Evans, 2002) and a voucher specimen was submitted to the Department of Pharmacognosy, Shri Ram Murti Smarak College of Engg and Technology Bareilly, UP (India).

The seeds were washed with fresh water to remove adhering dust, foreign particles and defective seeds. Excess of water was removed and the seeds were dried at 35-40^{0}C in an oven for 24 hours. The dried seeds were subjected to grind, the coarse powder mass was weighed and then allowed to undergo continuous heat extraction.

The powdered seeds of P. ovata (500 g) were extracted exhaustively with 95% ethanol in soxhlet apparatus. The ethanolic extract was concentrated to small volume then evaporated till dry. The % yield of P. ovata seed extract was 7.29%.

Chemicals

Aloe vera gel – (Dabur India Ltd, Nasik-422010,
Ketamine HCl – (Themis Medicare Ltd, Mumbai),
White Petroleum Jelly – (Central Drug house (P) Ltd, New Delhi).

Experimental Animals

Albino rats (Wister strain) of either sex weighing between 150-200 grams were kept in polypropylene cages at room temperature and 12/12 hours of light/dark cycles. The animal had free access to standard pellets and water under strict hygienic conditions. The animals were habituated to laboratory condition for 48 hours prior to experimental protocol to minimize, if any nonspecific stress. The study was approved by institutional ethical committee of Department of Pharmacy, Shri Ram murti Smarak College of Engg And Technology Bareilly, UP (India) regulated under CPCSEA with registration number 715/02/c/CPCSEA.

The rats were anaesthetized with Ketamine HCl (Dose 80 mg/kg IM) prior to and during infliction of the experimental wounds (Excision and incision). The surgical interventions were carried out under sterile conditions. After infliction of the wounds, the animals were randomly divided into six groups ((Excision model=3, Incision model=3) with six animals in each group.

Preparation of wound site by excision wound model

A circular seal of 2.5 cm uniform diameter was impressed on the shaved dorsal thoracic central region and the entire thickness of the skin from the marked area was excised to obtain a wound of about 500 mm^2 (Plate-1A) (Falanga, 2004, Ehrich and Hunk, 1969). The day of infliction of wound was considered as day '0'. After the infliction of wound, animals were housed individually in separate cages.

Wound area was measured by tracing the wound on a millimeter scale graph paper. The percentage of wound contraction was calculated of original wound size (500 mm^2) for each animal on predetermined days ie 4, 8 and 12th days of post wounding for final analysis of results (Kumar and Gupta, 2009). Animals were closely observed for any infection and those which showed signs of infection were separated and excluded from the study and replaced.

Creation of incision wound and tensile strength measurement

A 5 cm long abdominal incision was made in shaved area of anaesthetized rat and closed with interrupted sutures (Mersilk, Ethicon Aurangabad, Maharashtra) at a distance of 1 cm (Plate-2A) (Kumar and Gupta, 2009). They were housed individually in separate cages. On 12th day of post wounding, the animals were sacrificed by cervical dislocation and wound areas from each animal were removed carefully. Wound stripes of equal size (width) were cut and both ends of each strip were fixed with a pair of steel clip, one clip was allowed hanging on a stand and other clip with a polyethylene bag. It was then gradually filled with water till the wound strip was broken at the site of wound. The amount of water required to break the wound was noted and expressed as tensile strength of wound in grams (Kumar et al, 2010).

Acute dermal toxicity

The acute dermal toxicity was performed according to the OECD Guidelines (OECD 410). For this, a limit test of 2000 mg/kg did not show any sign of lethality or moribund state and the gross behavior of the animals was normal and there was no apparent sign of dermal toxicity.

Selection of dose and treatment period

P. ovata 10% ointment was used for topical application in excision and incision wound models. The treatment period was 12 days for excision and incision wound models. The day of infliction of wound was considered as day '0'.

Application of drugs for wound healing activity

Thirty six animals were divided into six groups ((Excision model =3, Incision model =3) with six animals in each group.

Excision model

Group 1: control, Group-2: test, Group-3: standard

Incision model

Group 4: control, Group-5: test, Group-6: standard

In the control group petroleum jelly was applied once daily for 12 consecutive days starting from day '0'. Similarly in test group and standard group P. ovata extract and Aloe vera gel (10%) was applied respectively once daily in both excision and incision wound model.

Statistical analysis

Results, expressed as mean ± SE were evaluated using the ANOVA. Values of $P<0.05$ were considered statistically significant.

RESULTS

The ethanolic extract of seeds of P. ovata (10% w/w) ointment had produced significant ($P<0.01$) increase in mean % wound contraction (22.39, 59.55 and 87.77%) after 4th, 8th and 12th day of treatment as compared with control (9.64, 53.69, 66.97) ((Table1) (figure1) (Plate1.A-1.D). Similarly a significant ($P<0.01$) increase in tensile strength (35%) on 12th day was observed more, as compared with control (Table1) (figure2) (Plate2A-2D). The wound contraction and tensile strength of experimental ointment under test were in close proximity with standard Aloe vera 10% cream.

Table 1: Effect of topical application of 10 % w/w ethanolic extract of seeds of *P. Ovata* on wound healing in rats.

Groups	% wound contraction in excision wound model			Tensile strength (g) in incision wound model
	4th day	8th day	12th day	
Control (white petroleum jelly)	9.64±2.1	53.69±6.0	66.97±1.4	286.75±27.15
Plantagoovata (10% w/w jelly)	22.39±1.5**	59.55±2.5*	87.77±3.2**	439.0±29.49**
Aloe vera (10% w/w gel)	23.80±6.2**	59.31±9.3*	90.27±3.7**	473.0±91.68**

All values are mean ± SD, *P< 0.05, **P<0.01, n=5.

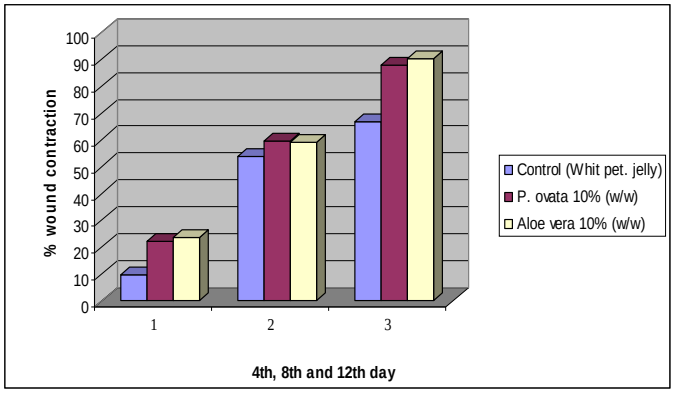
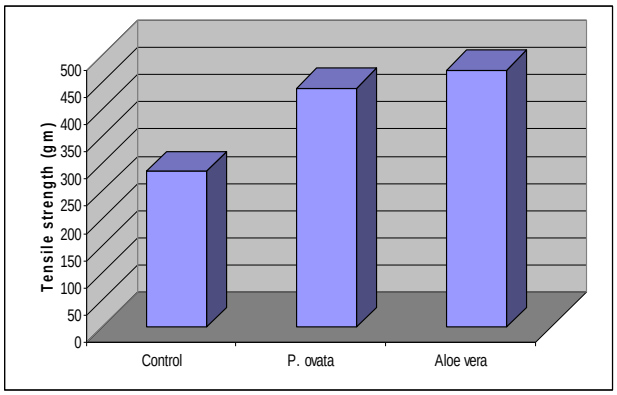


Fig 1: Effect of *P. ovata*seed extract on Excision wound.

Fig 2: Effect of *P. ovata* seed extract on Incision wound.



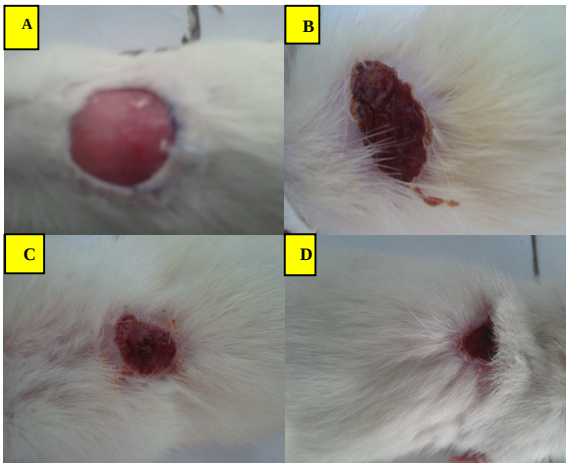


Fig 2: Plate 1- Excision wound healing: A. Excision wound on first day;B. Excision wound on 4th day; C. Excision wound 8th day;D. Excision wound on 12th day

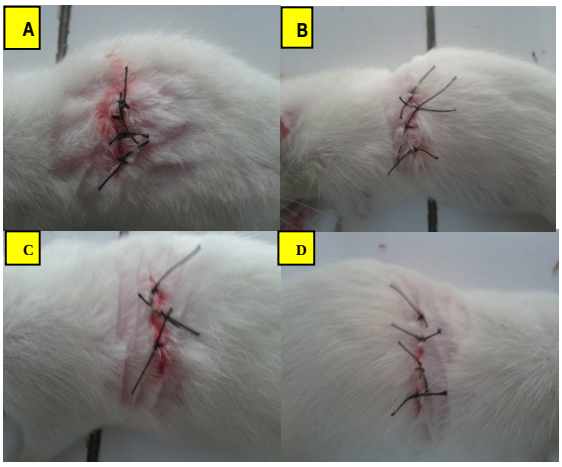


Fig 2: Plate 2- Incision wound healing: A. Incision wound on first day;B. Incision wound on 4th day; C. Incision wound 8th day;D. Incision wound on 12th day.

DISCUSSION

Wound healing is the primary response to tissue injury with different phases like contraction, granulation, epithelization and collagenation which is mainly achieved by connective tissue matrix synthesis (Pieree and Mustoer, 1995, Biswas and Mukherjee, 2003). Wound contraction is the process of mobilizing healthy skin surrounding the wound to cover the denuded area. This centripetal movement of wound margin is believed to be due

to the activity of myofibroblast (Gabbaiani et al, 1972). In our study, in excision wound model, the ethanolic extract of seeds of P. ovata (10% w/w) ointment has produced significant increase in mean % wound contraction after 4th, 8th and 12th day of treatment as compared with control group. This suggests that it would have either enhanced contractile property of myofibroblasts or increased the number of myofibroblasts recruited into the wound area.

The wound breaking strength is determined by the rate of collagen synthesis and more so by the maturation process where there is covalent binding of collagen fibrils through inter and intra molecular cross linking (Shanbhag et al, 2006). In the present study a significant increase in tensile strength on 12th day was observed in test group as compared with control group. This increase in tensile strength may be either because of increase in the collagen content or due to alteration in maturation process (by affecting the cross linking of collagen or improving the quality of collagen fibrils).

In recent years oxidative stress has been implicated in a variety of degenerative process and diseases. These include acute and chronic inflammatory condition (Maiere and Chan, 2002). An open label, multicenter, randomized clinical trial showed that P. ovata seeds might be as effective as mesalamine in maintaining remission in Ulcerative Colitis.[21] Some studies have also demonstrated the antioxidant activity of P. ovata (Fernández et al, 1999, Rodríguez et al, 2002). In addition to this phytochemical screening has revealed the presence of flavonoids in P. ovata which are known free radical scavengers (Marina et al, 2003). This might be the other reason for the wound healing activity of P. ovata.

CONCLUSION

The ethanolic extract of seeds of P. ovata applied topically, effectively increased (87%) the contraction of open wound, tensile strength (35%) after 12 days which was significant as compared to control ($P<0.01$). The results of the study suggest that P. ovata has significant wound healing activity in both excision and incision wound model and it could possibly be made use of clinically, in healing of open wounds. However, implementation of these results need well designed clinical evaluation.

ACKNOWLEDGEMENT

Authors are thankful to Mr Dev Murti, Chairman, SRMS College of Engineering & Technology, Bareilly for his encouragement and providing research facilities and SRMS trust for financial support.

REFERENCES

Biswas T.K., Mukherjee B. Plant medicines of Indian origin for wound healing activity: A review. Int J Lower Extremity Wounds. 2003; 2(1): 25-39.

Charde R.M., Charde M.S., Fulzele S.V., Satturwar P.M., Joshi S.B. Wound healing activity of ethanolic extract of Rubia cordifolia roots. J Phar Res. 2010; 3(12): 3061-3063.

Ehrich H.P., Hunk T.K. Effect of cortisone and anabolic steroids on tensile strength of healing wound. Ann Surg. 1969; 170: 203-206.

Evans, W.C. (2002). Psyllium. In Trease and Evans (Ed.) Pharmacognogy. (pp. 210). Sounder WB Company Limited.

Falanga V. The chronic wound: Impaired wound healing and solution in the context of wound bed preparation, blood cells mole. Disease. 2004; 32(84): 88-94.

Fernandez B.F., Hinojosa J., Sanchez L.J.L., Navarro E., Martinez S.J.F., García P.A., Gonzalez H.F., Riera J., González L.V., Domínguez A.F., Gine J.J., Moles J., Gomollón F., Gassull M.A. Randomized mesalamine in maintaining remission in ulcerative colitis. Spanish Group for the Study of Crohn's Disease and Ulcerative Colitis (GETECCU). Am J Gastroenterol. 1999; 94: 427–433.

Gabbaiani G., Harschel B.J., Ryan G.B. Granulation tissue as a contractile organ. J Exp Med. 1972; 135(4): 719-734.

Gupta N., Jain U.K.. Prominent wound healing properties of indigenous medicines. J Nat Pharm. 2010; 1(1): 2- 5.

Hannan J.M.A., Ali L., Khaleque J., Akhter M., Flatt PR., Wahab A. Aqueous extracts of husks of Plantago ovata reduce hyperglycaemia in type 1 and type 2 diabetes by inhibition of intestinal glucose absorption. Br J Nut. 2006; 96: 131–137.

Holla R.K., Sequeira R.P., Kulkarni D.R.. Cyclosporin and wound healing. Ind J Exp Biol. 1988; 26: 869– 873.

Isabgol cultivation- A profitable cultivation, accessed on date 20/06/2011 http://www.agricultureinformation.com /photos/showphoto.php photo=61

Kumar N., Gupta A.K.. Wound-Healing Activity of Onosma hispidum (Ratanjot) in Normal and Diabetic Rats. J Herbs Spices Med Plants. 2009; 15(4): 342-351.

Kumar N., Prakash D., Kumar P. Estimation of Wound healing activity of Solanum xanthocarpum Schrad & Wendl. Fruits. Ind J Nat Pro Reso. 2010; 1(4): 470-475.

Lee K.H. Studies on the mechanism of action of salicylates III. Effect of vitamin A on the wound healing retardation action of aspirin. J Pharma Sci. 1968; 57(7): 1238–1240.

Maiere C.M., Chan P.H. Role of superoxide dismutase in oxidative damage and neurodegenerative disorders. Neuroscientist. 2002; 8(4): 323–334.

Marina G., Carmen M.C., Miguel L.L., Felipe C., Mar´ıa J.A. Cytotoxic effect of Plantago spp. on cancer cell lines. Journal of Ethnopharmacology. 2003; 88: 125–130.

Pieree G.F., Mustoer T.A. Pharmacologic enhancement of wound healing. Annu Rev Med. 1995; 46: 467-481.

Prasad D., Rao C.M. Wound healing profile of ketorolac, metronidazole and tinidazole administered postsurgically. Ind J Exp Biol. 1995; 33: 845–847.

Raju S., Kulkarni D.R. Vitamin A reverses the wound healing suppressant effect of cyclophosphamide. Ind J Pharmacol. 1986; 18: 154–157.

Rao C.M., Ramesh K.V., Bairy K.L., Kulkarni D.R. A simple method to quantify maturation of wound collagen. Ind J Exp Biol. 1991; 29: 156–158.

Rodríguez C.M.E., Gálvez J., Lorente M.D., Concha A., Camuesco D., Azzouz S., Osuna A., Redondo L., Zarzuelo A. Dietary fiber down-regulates colonic tumor necrosis factor alpha and nitric oxide production in tri nitro benzene sulfonic acid-induced colitic rats. J Nutr. 2002; 132(11): 3263–3271.

Rodríguez C.M.E., Gálvez J., Camuesco D., Lorente M.D., Concha M., Augustin O.M., Redondo L., Zarzuel A. Intestinal anti-inflammatory activity of dietary fiber (Plantago ovata seeds) in HLA-B27 transgenic rats. Clin Nutria. 2003; 22(5): 463-471.

Shanbhag T.V., Sharma C., Sachidananda A., Bairy L.K., Shenoy S., Shenoy G. Wound healing activity of alcoholic extract of Kaempferia galanga in wistar rats. Indian J Physiol Pharmacol. 2006; 50 (4): 384–390.