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Research Protocol

Complementary/Alternative Medicine Section

Assessment of *Vranaropana* (Wound Healing) Activity of *Porana paniculata* Roxb. in Wistar Albino Rats: An Experimental Study Protocol

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ABSTRACT

Introduction: Ethnomedicine is the study of medicinal plants and their traditional uses in treating a variety of illnesses. Research on ethnomedicine and ethnobotany has great potential to grow and produce new medications. Because medicinal plants include a variety of biologically active compounds, documenting ethnomedicinal information is crucial to the discovery of novel molecules based on traditional knowledge.

Need of the study: The plant *Porana paniculata* Roxb. has Ethno-medicinal properties. Traditional healers in the state of Chhattisgarh use its root paste topically to treat wounds and cure bone fractures. Only a single paper has been published on its anti-inflammatory and analgesic properties. Its ability to cure wounds has not been the subject of any scientific research.

Aim: The study aims to evaluate the wound-healing activity of the extract of the root of *P.paniculata* Roxb. in Wistar albino rats.

Materials and Methods: An experimental study will be conducted at Datta Meghe College of Pharmacy and Research, Datta Meghe Institute of Higher Education and Research, Sawangi (Meghe), Wardha, Maharashtra, India from June 2024 to Dec 2024. The Botanical Survey of India (BSI) will verify the authenticity of *P.paniculata* Roxb. roots that will be obtained from Chhattisgarh in *Grishma* (mid-April to mid-June) or *Shishira Ritu* (mid-December to mid-February). Wistar albino rats, scientific-grade chemicals, and gram-positive and gram-negative microorganisms will all be purchased from reliable sources. The preparation of an extract ointment will be employed to evaluate the activity of wound healing, in Wistar albino rats utilising an excision wound model. The antimicrobial activity will be evaluated using an Agar well diffusion technique. Data obtained will be analysed using One-way ANOVA and a p-value less than 0.05 will be considered as significant.

Keywords: Antimicrobial, Bridal bouquet, Ethnobotany, Phytochemical analysis

INTRODUCTION

There isn't a medication in the market that can speed up wound healing. Medicines from Ayurveda may speed up wound healing. Since many plant extracts and phytoconstituents have a variety of potent mechanisms, are readily available, and have few adverse effects, they can be employed as a viable alternative for wound healing [1]. Traditional medicine is well-ingrained and has a very long history in India. Up to 80% of people worldwide receive their primary medical treatment from conventional medicine, according to the World Health Organisation. The study of medicinal plants and their traditional usage in various parts of India has gained more attention in the past few decades, and there are several accounts of the use of plants in traditional healing by Indian communities and tribes [2]. Due to their deep belief in these systems and, to some extent, the absence of adequate and trustworthy healthcare facilities, traditional medicines play a significant role in the primary healthcare of Indigenous people living in remote tribal settlements. Tribes and forest inhabitants make up a sizable portion of the population in Chhattisgarh. A large number of medicinal plants find refuge in Chhattisgarh, which has 44% of its land covered in forests with great plant species diversity. Numerous tribal tribes reside in isolated, inaccessible forest regions. Traditional healers primarily obtain their medicine from the local plant resources. Because tribal and forest people live near nature, they have learned how to make use of the plants that grow there [2]. Many ethnobotanical survey studies have been carried out in several regions of Chhattisgarh, including Belgahana block of Bilaspur, Raipur [3], Bharatpur block of Koriya [4], Dantewada [5], Jashpur [6], Baster [7], Raigarh [8], and Sarguja [9]. Many ethnomedicinal plants are accessible in India and are used in many forms for wound treatment, including paste, juice, decoction, and powder, with portions including roots, leaves, and stems employed.

Wound healing is a difficult procedure. It begins at the point of wounding and lasts until full recovery. Healing time is based on the size of the wound. There are three stages involved in the healing of wounds: the inflammatory, proliferative, and healing phases. A good wound healing agent promotes fibroblast proliferation, induces keratinocyte proliferation and differentiation, increases collagen synthesis, and demonstrates anti-microbial, anti-oxidant, and anti-inflammatory properties [10]. This is caused by a variety of phytochemicals, including terpenoids, flavonoids, and phenolics [11]. The majority of medications with wound-healing action also exhibit antimicrobial activity [12]. *P.paniculata* is not a much-explored drug. There is a dearth of published literature on this subjects. This research is being planned to learn more about this medication. Hence, the study aimed to evaluate the wound-healing properties of the drug *P.paniculata* Roxb.

Primary Objectives

1. To study wound healing activity of *P.paniculata* Roxb. in Wistar Albino rats.
2. To study the In vitro Antimicrobial activity of *P.paniculata* Roxb.

Secondary Objectives

1. To compare the wound healing activity of *P.paniculata* with the standard drug Betadine.
2. To study the dermal toxicity study of *P.paniculata* Roxb.
3. To assess wound healing activity by histological examination.

REVIEW OF LITERATURE

P.paniculata Roxb. is a robust, shrubby climber that bears many white flowers in panicles that end each branchlet. 'The bridal bouquet' is its popular name in English, and it belongs to the convolvulaceae family. Synonyms for this are *Porana tomentosa* Leschen, *P.paniculata Roxburgh*, and *Dinetus paniculatus* (Roxburgh).

P.paniculata has been used to treat pain and inflammation in folk medicine [13]. Its whole plant extract showed anti-inflammatory and analgesic activity by a hot plate, tail immersion, acetic acid-induced writhing model, and carrageenan, histamine-induced model, respectively [14]. Traditional healers in Chhattisgarh employ *P.paniculata* to treat wounds and fractures. For this, root paste is applied locally. It is an excellent medication for healing fractures and wounds. It is known as "Masbandhi" in the Chhattisgarhi language, meaning that it will bond the flesh [10].

Numerous study publications in ethnobotany have documented *P.paniculata* Roxb. as a plant used in folklore [13-15]. On its anti-inflammatory and analgesic properties, only a single study using the entire plant extract has been published [15]. A retrospective study was conducted by Kumar AS et al., to evaluate the preliminary phytochemical, analgesic, and anti-inflammatory activities of the *P.paniculata* whole plant. Plant material was subjected to extraction by maceration using ethanol and water mixture as a solvent and subjected to preliminary phytochemical screening. For analgesic activity, hot plate, tail immersion, and acetic acid-induced writhing models were used whereas for anti-inflammatory activity, carrageenan and histamine induced model were employed. *P.paniculata* stem bark ethanolic extract showed antioxidant activity while Phytochemical screening showed the presence of alkaloids, flavonoids, phenolics, carbohydrates, reducing sugars, terpenoids, steroids, and tannins [15].

MATERIALS AND METHODS

An experimental study will be conducted at Datta Meghe College of Pharmacy and Research, Datta Meghe Institute of Higher Education and Research, Sawangi (Meghe), Wardha, Maharashtra, India from June 2024 to Dec.2024. The approval has been obtained from IAEC, protocol number DMIMS/IAEC/2020-21/26, and issued on May 10, 2021. Plant material i.e., roots of *p.paniculata* Roxb. will be collected from Chhattisgarh in *Grishma* (mid-April to Mid-June) or *Shishira Ritu* (Mid December to mid-February) as said by *Acharya Charaka*. An interview of the traditional healer will be done and the plant will be identified by him. This will be authenticated by the BSI. Reagents and chemicals of the analytical grade of Merck Company will be procured from an available authentic source. The standard pathogenic bacteria culture Gram-positive *Staphylococcus aureus* ATCC no.80 cc, *Bacillus subtilis*, and Gram-negative *Pseudomonas aeruginosa* ATCC No.88, *Escherichia coli* will be procured from available authentic sources. A total of 24 healthy Wistar Albino Rats of both sexes weighing 180-200 gm will be procured from Central Preclinical Research Facility, Datta Meghe College of Pharmacy, Sawangi (Meghe), Wardha, and included in the study.

Diseased animals having weight less than 180 gm and more than 200 gm will be excluded.

The animals will be housed in an environment with a controlled temperature and humidity level (25±0.50C) and a 12-hour light/dark cycle. Water will be provided freely to the animals along with a regular pellet feed.

Sample size: There will be three groups, each with six animals. A simple randomisation method will be used to assign animals to groups using a random number generator. The experimental group will receive treatment with an experimental drug i.e., *P.paniculata* extract ointment, control group will receive treatment with an ointment base (glycol stearate, propylene glycol, and liquid paraffin in the ratio of 3:6:1) and the Standard group will receive treatment with a conventional medicament i.e., Betadine ointment. The 0.5 gm dose of ointment will be used topically once a day until the wound heals completely. In the present study, no animal will be sacrificed. The sample size for each group will be six animals as per standard animal study guidelines for acute dermal toxicity study and wound healing activity study [16]. Wound healing will be assessed based on wound size contraction, epithelisation period, and histopathological examination. Anti-microbial activity will be assessed by Minimum Inhibitory Concentration (MIC). The limit test for the acute dermal toxicity investigation will be created using OECD guideline no. 434 and administered using a fixed-dose technique to 06 female Wistar rats. Dermal toxicity will be assessed by changes in skin, like the presence of redness, erythema, blisters, discharge, and haemorrhage.

Ointment preparation: An ointment base will be prepared using a 3:6:1 ratio of glycol stearate, propylene glycol, and liquid paraffin. Ten grams (10% w/w) of *P.paniculata* root extract will be added to one hundred grams of ointment base [17]. Betadine ointment will be used as a standard drug to compare wound healing potential with an extract of *P.paniculata*. The 0.5 gm dose of ointment will be used topically once a day until the wound heals completely.

Anti-microbial study: The antimicrobial effect will be estimated using the Agar well diffusion technique [18]. The extract will be given at a 50 mg/mL dosage after being dissolved in Dimethyl Sulphoxide (DMSO). Using the serial dilution approach, the MIC will be determined. The extract will be utilised at the following concentrations: 25.0, 12.5, 6.25, 3.125, 1.562, 0.781, and 0.39 mg/mL. ciprofloxacin will be employed as a common antibacterial. Only DMSO will be present in the control agar well.

Animal Study for Wound Healing Activity

Wound induction: An excision wound model will be used. Each animal in every group will get an injection of ketamine hydrochloride (50 mg/kg body weight) to induce unconsciousness. The rats' dorsal thoracic region will be shaved, and a full-thickness excision wound spanning 500 mm^2 will be made along the marking using toothed forceps, a surgical blade, and pointed scissors. This will be done from a pre-selected location on the rats' dorsal back, 1 cm from the vertebral column, and 5 cm from the ear [17].

Outcome Measures

Wound contraction: The area of the wound will be estimated right away by tracing it on clear paper and computing the area using a graph sheet measuring 1 mm. Every third day until full healing, the same methodology will be used to measure wound contraction. We'll compute the proportion of wound contraction. The wound's area is measured as 100% at the moment of wounding, and the day of wounding is measured as 0% [17].

$$\% \text{ wound contraction} = \frac{\text{Initial wound area-specific day wound area} \times 100}{\text{Initial wound area}}$$

Epithelisation period: The number of days needed for the wound area to fully heal and shed its crust will be used to estimate the epithelisation duration. Using a digital camera to take digital photos of the skin and wound, the macroscopical assessment of the healing process will be conducted.

Histological examination: A histological examination will be done to assess the wound healing. A Punch biopsy will be done on the 21st day after wounding to gather samples as per research done by Lemo N et al., [19]. Formalin with a 10% neutral buffer will be used to repair the sample. Staining with Masson's Trichrome, Haemotoxylin and Eosin (H&E) will be used for the histological evaluation [19].

Acute Dermal Toxicity Study

The limit test for the acute dermal toxicity investigation will be created using OECD guideline no. 434 and administered using a fixed-dose technique to 06 female Wistar rats. Weighing and measuring the body surface area of each animal will be done. A 10% of the total

body surface area shall be thoroughly shaved, and the shaved area will be topically coated with extract ointment (2000 mg/kg body weight). The animals will be monitored for signs of toxicity for twenty-four hours straight, and then periodically, for the following thirteen days [16].

STATISTICAL ANALYSIS

Statistical analysis will be done by using SPSS software, and MS Office Excel. Data obtained will be analysed by applying the One-way Analysis of Variance (ANOVA). Tukey's HSD post hoc analysis will be applied for inter-group comparison. The p-value less than 0.05 will be considered as significant.

REFERENCES

[1] Dev SK, Chaudhary PK, Shrivastava R, Sharma M. Antimicrobial, anti-inflammatory and wound healing activity of polyherbal formulation. Biomed Pharmacother. 2019;111:555-67.
[2] Husain N, Chauhan D, Trak TH. Some ethno-medicinally important plants from Chhattisgarh, India, with caution stigma about their abortifacient activity. International Journal of Science and Research. 2017;6(7):2046-51.
[3] Dewangan K. Ethnobotanical survey of Raipur Chhattisgarh with special reference to plants described in folklores and used in festivals (Synopsis). Shodhganga. 2019;301.
[4] Sinha MK. Ethnomedicinal plants used in Tribes of Bharatpur block (Koriya district) C.G. Journal of Medicinal Plants Studies. 2017;5(4):17-21.
[5] Sahu PK, Masiha V, Gupta S, Sen D, Tiwari A. Ethnomedicinal plants used in the healthcare systems of tribes of Dantewada, Chhattisgarh India. American Journal of Plant Sciences. 2014;5:1632-43.
[6] Sharma RK. Study of ethnomedicinal of plants used for various ailments of Bagicha Jashpur (C.G.) India. European Journal of Biotechnology and Bioscience. 2017;5(6):41-43.
[7] Mahant SD. Indigenous traditional healing care: Belief & practices among tribals of South Bastar in Chhattisgarh. IOSR Journal of Humanities and Social Science. 2015;20(1):49-54.
[8] Singh U, Bharati AK. Ethnobotanical study of plants of Raigarh Area, Chattisgarh, India. Int Res J Biological Sci. 2015;4(6):36-43.
[9] Shrivastav S, Kanungo VK. Ethnobotanical survey of Surguja district with special reference to plants used by Uraon tribe in treatment of respiratory diseases. International Journal of Herbal Medicine. 2013;1(3):131-34.
[10] Dwivedi D, Dwivedi M, Malaviya S, Singh V. Evaluation of wound healing, anti-microbial and antioxidant potential of Pongamia pinnata in Wistar rats. J Tradit Complement Med. 2017;7(1):79-85.
[11] Sharma T, Bhide B, Acharya A. Wound healing activity of certain root drugs-A review from the classical and ethnomedicinal claim. I J Green Pharmacy. 2019;13(1):21-41.
[12] Juneja K, Mishra R, Chauhan S, Gupta S, Roy P, Sircar D. Metabolite profiling and wound-healing activity of Boerhavia diffusa leaf extracts using in vitro and in vivo models. J Tradit Complement Med. 2020;10(1):52-59.
[13] A SK, Juturu RR, Vankadari RM. Effect of porana paniculata whole plant on blood glucose levels and lipid profile of STZ induced diabetic rats. Pharmacognosy Journal. 2019. Available from: https://phcogj.com/article/870. Accessed on 10/04/2024.
[14] Peng Y, Li Y, Yang Y, Gao Y, Ren H, Hu J, et al. The genus Porana (Convolvulaceae) - A phytochemical and pharmacological review. Front. Pharmacol. 2022;13:998965. Doi: 10.3389/fphar.2022.998965.
[15] Kumar AS, Reddy JR, Gupta VRM. Evaluation of analgesic and anti inflammatory activities of Porana paniculata whole plant. International Journal of Pharma and Bio Sciences. 2014;5(4):P-413-P-421.
[16] OECD guideline for the testing of chemicals Acute Dermal Toxicity: Fixed Dose Procedure 9789264070585-en.pdf (oecd-ilibrary.org). Accessed on 19/07/2024.
[17] Güzel S, Özay Y, Kumaş M, Uzun C, Özkorkmaz EG, Yıldırım Z, et al. Wound healing properties, antimicrobial and antioxidant activities of Salvia kronenburgii Rech. f. and Salvia euphratica Montbret, Aucher & Rech. f. var. euphratica on excision and incision wound models in diabetic rats. Biomed Pharmacother. 2019;111:1260-76.
[18] Holder IA, Boyce ST. Agar well diffusion assay testing of bacterial susceptibility to various antimicrobials in concentrations non-toxic for human cells in culture. Burns. 1994;20(5):426-29.
[19] Lemo N, Marignac G, Reyes-Gomez E, Lilin T, Crosaz O, Ehrenfest DMD. Cutaneous reepithelialization and wound contraction after skin biopsies in rabbits: A mathematical model for healing and remodeling index. Veterinarski Arhiv. 2010;80:637-52.

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