

WOUND HEALING ACTIVITY OF *PROSOPIS JULIFLORA* LINN.**Thirupathy Kumaresan P^{*}, Deepa M.¹, SirrajudeenA.¹, Perumal P**^{*}Nirmala College of Pharmacy, Muvattupuzha, P.O Kerala-686 661.¹Department of Pharmacology, A.K.College of Pharmacy, Krishnankoil-626126.Article Received on
08 July 2015,Revised on 31 July 2015,
Accepted on 24 Aug 2015***Correspondence for
Author****Thirupathy Kumaresan P.**
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Pharmacy, Muvattupuzha,
P.O Kerala-686 661.**ABSTRACT**

Prosopis juliflora is used in herbal medicine for the treatment of various diseases such as infection, pain, inflammation and wounds. The pet - ether, benzene, ethyl acetate and alcoholic extracts of *Prosopis juliflora* flowers were subjected to preliminary qualitative chemical investigation. Phytochemical analysis of extracts revealed that the presences of steroid, alkaloid, saponin, flavonoid and tannin in the flower parts of *Prosopis juliflora*. LD₅₀ values were determined based on OCED guidelines for three extracts. The extracts did not produce any mortality even at 5000 mg/kg. The effect of pet - ether, benzene ,ethyl acetate and alcoholic extract of *Prosopis juliflora*

flowers were investigated in rats to evaluate wound healing activity by using three models i.e incision, excision and dead space (granuloma). From the results obtained it may be concluded that ethyl acetate extract of *Prosopis juliflora* flowers exhibited more significant wound healing activity than other extract when compared to the reference drug ointment.

KEYWORDS: Wound healing activity, *Prosopis juliflora*. Hydroxyproline.**INTRODUCTION**

Wound healing is a biochemical process and a cellular event occurs in three stages like inflammation, proliferation, and remodeling which designed to repair tissue integrity following injury.^[1] Initial stage of this process involves an acute inflammatory phase followed by synthesis of collagens and other extracellular macromolecules which are later remodeled to form scar.^[2, 3] *Prosopis juliflora* is a small tree (family: Mimosacea) commonly known as Club moss. The plant is found distributed all along the dry area of all over India. It has been used as a folk remedy for anemia^[4], catarrh, cold, diarrhea, dysentery, inflammation, measles, sore throat and in healing of wounds.^[5]

The alkaloids and flavones glycoside i.e Patulitrin, Procyanidin, ellagic acid, tannin and polystyrenes have been isolated from leaf extracts of *Prosopis juliflora*^[6,7 8] Several natural products like plant products which are composed of active principle such alkaloids and flavonoids have been reported to promote the process of wound healing.^[9,10] In view of these cited activities, the use of traditional and observation, the present study was undertaken to explore the wound healing potential of various extracts of *Prosopis juliflora* flowers.

MATERIALS AND METHODS

The flower parts of *Prosopis juliflora* were collected from the Western Ghats range of Virudhunagr district of Tamil Nadu State, during the month of September to October 12 and voucher specimen (BKM-456) was authenticated by referring the specimen to Botanical survey of India and Agriculture University Coimbatore and a voucher specimen was deposited in the department of Pharmacology, A.K.College of Pharmacy, Krishnankoil for future reference. The flowers of *Prosopis juliflora* were shade dried and powdered mechanically (sieve size 10/45). About 1 kg of powdered material was exhaustively extracted with 60 – 80 ° C petroleum ether, benzene, ethyl acetate and alcohol for about 72 h in a Soxhlet extractor. The extract was filtered and concentrated in vacuum under reduced pressure using rotary flash evaporator (Buchi, Flawil, Switzerland) (yield 10.6 %, 13.6 %, 20.8 % 21.6 % w/w respectively). All the extracts were subjected to preliminary phytochemical screening as described by methods of Harbone.^[11] For topical administration, 5 % w/w ointment was prepared with different extracts incorporate in 2 % simple ointment base.

Animals

Wistar rats of either sex weighing 180-220 g and male albino mice (25 -28 g) were procured from animal house from A.K. College of Pharmacy, Krishnankoil, were maintained at standard housing conditions. The animals were fed with commercial diet (Hindustan Lever Ltd., Bangalore) and water *ad libitum* during the experiment. The animal experiments were conducted as per protocol approved (approval no 123/2003/c/CPCSEA) by Institutional animal ethics committee (IAEC) and as per Indian norms laid down by committee for the purpose of control and supervision of experimental animals (CPCSEA), New Delhi.

Acute toxicity study

Male Swiss albino mice (25 g- 28 g) were dosed with extract and were observed for any symptoms of toxicity for 48 hrs as per OCED guidelines 425 and LD₅₀ was estimated using

AOT 425 software (Westat, EPA, USA). Based on the results obtained from this study, dose for the further pharmacological studies was fixed to be 200 mg/kg.^[12]

Wound healing activity

The rats were inflicted with excision wounds as described by Morton and Malone^[13] under light ether anesthesia. A circular wound of about 500 mm² was made on depilated ethanol sterilized dorsal thoracic region of rats. The wound was left undressed to open environment. The animals were divided into 6 groups of 6 each. The group I was considered as the control and received simple ointment base, the group II received 0.2 % ointment served as reference standard, group III, IV, V, and VI animals were received pet ether, benzene, ethyl acetate and alcoholic extract (5 % w/v) ointments respectively. The ointment was topically applied once in a day till the epithelisation was complete from the day of the experiment. The parameters studied were percentage closure of excision wound and epithelisation time. The wounds were traced on sqmm2 graph paper on 4th, 8th, 12th, and 16th day and thereafter on alternative days until healing was completed. The percentage of wound closure and the period of epithelisation were recorded.^[13]

In incision wound model^[13] two para vertebral straight incision of 6 cm each was made through the entire thickness of skin on either side of the vertebral column with the help of surgical blade anaesthetized animals after complete hemostats the wounds were closed by means of interrupted sutures at equidistant points about 1 cm apart using four zero silk thread and surgical curved needle. The animals were divided into six groups of six animals was similar to that of excision wound model. Simple ointment base (control) Povidone ointment (0.2 % w/w reference standard), pet-ether, benzene, ethylacetate and alcoholic extract (5 % w/w) ointment were applied once in day for seven days. Removal of sutures was done on 8th post wounding day and possible strength of the healed wound was measured on 10th post wounding day by continuous water flow technique.^[13]

Dead Space Wound Model

Dead space wounds were inflicted by implanting sterile cotton pellets (5 mg each), one on either side of the groin and axilla on the ventral surface of each rat by the technique of D'Arcy *et al* as described by Turner. The animals were randomly divided into six groups of six each. The control group animals were provided with plain drinking water. The test group rats were given extract orally in their drinking water at a dose of 200 mg kg⁻¹ daily. On the 10th post wounding day, the granulation tissue formed on the implanted cotton pellets was

carefully removed under anesthesia. The wet weight of the granulation tissue collected was noted. These tissues samples were dried at 60 °C for 12 h and weighed to determine the dry granulation tissue weight. Dried tissue was added with 5 ml 6 N HCl and kept at 110 °C for 24 h. The neutralized acid hydrolysate of the dry tissue was used for the determination of hydroxyproline.^[14, 15]

Statistical analysis

The data were subjected to ANOVA followed by Turkey's multiple comparison test and the values of $P < 0.01$ were considered statistically significant.

RESULTS

Phytochemical screening of all the extracts gave positive test for steroids, alkaloids but ethyl acetate extract only gave positive test for flavonoid and tannins. In excision wound model, wound contraction was 86.24, 92.14, 98.24, 82.82 and 96.45 respectively on 16th day pet ether, benzene, ethyl acetate, alcoholic extract and Povidone treated groups (Table 1). Significant wound contraction was observed on 16th day for all treated groups. Time for complete epithelization significantly short in tested drug and standard drug treated groups. The ethyl acetate extracts treated animals showed faster epithelialisation of wound (17.18 ± 0.21). The period of epithelialisation was 24.21 ± 0.61 in case of control. The treated animals showed significant wound healing activity when compared to control (Table-1). In incision model, significant increase in tensile strength of healed wounds was observed in Povidone treated group (602 ± 13.2) and ethyl acetate treated group (594 ± 13.4) (Table – 2). In dead space wound model, topical application pet ether, benzene, ethyl acetate and alcohol extracts significantly increase dry weight of granulation tissue (65.21 ± 1.31 , 59.42 ± 1.01 , 59.61 ± 1.12 , 63.42 ± 1.42 and 59.38 ± 1.34), tissue breaking strength (345.24, 295.71, 294.21, 320.42 & 296.62) and hydroxyproline content (47.24, 30.21, 38.82, 30.72) respectively compared to control (dry weight of granulation tissue 58.46, tissue breaking strength 282.64, hydroxyproline content 26.45). Among tested extracts, ethyl acetate extract was found to be better wound healing activity. The wound area measurement showed that the wound size of the test groups was reduced early as compared to control groups. The results offer pharmacological evidence on the folkloric use of *Prosopis juliflora* flowers for healing wounds.

Table I Effect of topical application of different extracts of *Prosopis juliflora* flowers on excision wound in rats.

Group	4 th day	8 th day	12 th day	16 th day	Mean time of epithelization in days
Control Simple ointment base	21.24±0.32	44.12 ± 0.42	66.23 ± 0.42	82.62 ± 0.31	24.21 ± 0.61
Pet. Ether extract Ointment(0.5 % w/w)	28.12±0.82	48.21 ± 1.12	67.14 ± 1.02	86.24 ± 0.86	21.21 ± 0.32
Benzene extract Ointment (0.5 % w/w)	25.24±0.64	29.82 ± 0.162**	73.72 ± 0.72**	92.14 ± 0.82**	16.84 ± 0.28**
Ethyl Acetate extract ointment (0.5 % w/w)	28.62±0.82**	56.84 ± 0.86***	82.62 ± 0.56***	98.24 ± 1.02***	17.18 ± 0.21**
Alcohol extract ointment (0.5 % w/w)	26.11±0.84*	42.42 ± 1.24*	69.12 ± 1.02*	82.82 ± 0.81*	19.22 ± 0.24*
Povidone ointment (0.5 % w/w)	25.96±0.34**	58.25 ± 0.56***	78.71 ± 0.58***	96.45 ± 1.04***	16.23 ± 0.32***

All values are expressed in mean ± SEM; N=6 animals in each group

* Significant difference at P < 0.1 when compared to control

** Significant difference at P < 0.01 when compared to control

*** Significant difference at P < 0.001 when compared to control

Table II Effect of topical application of different extracts of *Prosopis juliflora* flowers ointment on Incision wound in rats

S.No	Group	Tensile strength in gram Mean ± SEM
1	Simple ointment base (Control)	422 ± 11.22
2	Povidone ointment (Ref. Std) (0.2% w/w)	602 ± 13.2*
3	Pet- Ether extract ointment	542 ± 12.1*
4	Benzene extract ointment	562 ± 12.2*
5	Ethyl Acetate ointment	594 ± 13.4**
6	Alcohol extract ointment	552 ± 12.1*

Values are mean ± SEM of 6 animals in each group

* p < 0.001 vs Control

** p < 0.01 vs respective control by student t' Test.

TABLE – III: Effect of oral administration of different extracts of *Prosopis juliflora* flowers on Dead Space Wound model

Group	Breaking strength (gm) mean ± SEM	Granulation tissue Dry weight (mg/100 gm) mean ± SEM	Hydroxy proline content – µgm /100 mg mean ± SEM
Control	282.64 ± 8.12	58.46 ± 1.21	26.45 ± 0.76

Povidone	345.24 ± 10.21	65.21 ± 1.31	47.24 ± 1.21
Pet-Ether extract	295.71 ± 8.24	59.42 ± 1.01	30.21 ± 0.56*
Benzene extract	294.21 ± 7.34	59.61 ± 1.12	38.82 ± 0.76*
Ethyl acetate extract	320.42 ± 11.31	63.42 ± 1.42	38.72 ± 0.62*
Alcohol extract	296.62 ± 12.11	59.38 ± 1.34	30.71 ± 0.45*

N = 6 animals in each group, Values are expressed as mean ± SEM, * P < 0.01 vs control

DISCUSSION

The wound healing process differs from tissue to tissue. In this discussion, skin is a protective for internal organs. Different types of wounds involve different steps of the healing process to varying degrees, although the steps themselves remain the same. In the present study, the phytochemical investigation of flowers extracts showed the presences of triterpenoids, saponins, alkaloids and flavonoids. Several phytoconstituents like triterpenoids, saponins^[16], alkaloids^[17] and flavonoid^[18] are known to promote wound heal process due their antioxidant and antimicrobial activities. In addition triterpenoids reported to possess an ability to increase the collagen content, which is one of the factors promoting wound healing.^[19] Furthermore the wound healing effects can be attributed to free radical scavenging activity of flavonoids and triterpenoids. Both these class of phytoconstituents are known to reduce lipid per oxidation, not only by preventing or slowing the onset of cell necrosis, but also improving vascularity. Lipid per oxidation is an important process in several types of injuries like burns, infected wound, skin ulcer etc., Hence any drug that inhibits lipid per oxidation is believed to increase the viability of collagen fibrils, which in turn results in increase in the strength of collagen fiber by increasing the circulation preventing the cell damage and promoting the DNA synthesis.^[20] This is suggested that there was an increase in the wound breaking and granuloma breaking strength after the administration of different extracts out of different tested extracts an ethylacetate extract endowed with significant wound healing activity and even comparable with standard drug (Povidone ointment). Flavonoid reduces lipid per oxidation by preventing or slowing the onset of cell necrosis and by improving vascularity.^[17] The wound healing process involves various phases viz., granulation, wound contraction, collagenation, collagen maturation and scar formation.^[22] Measurement of the hydroxyproline could be used as an index for collagen turnover.^[23] In the present study significant increase in the hydroxyproline content of the granulation of the animals treated with ethyl acetate extract and alcohol extract were observed indicating rapid collagen turnover. Increase in breaking strength of granulation tissue indicated the enhanced collagen maturation by increased cross linking. Thus, the observed wound healing activity of title

plant may be attributed to the presence of these bioactive principle and their synergistic properties. We have been concluded that the topical administration of various extract of PJ in the form of ointment plays major role in wound healing activity. It may be the presences of alkaloid, teriphenoid and flavonoid in the extracts.

ACKNOWLEDGEMENT

The authors are grateful to Kalvi vallal Thiru T.Kallasalingam B.com., and Iaya vallal Sri.K.Sridharan M.B.A, M.Phil (A.K. College of Pharmacy, Krishnankoil) for providing the facilities to carry out our researchwork successfully.

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