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EVALUATION OF WOUND HEALING ACTIVITY OF ZIZIPHUS XYLOPYRUS (RETZ) WILLD LEAVES EXTRACT IN WISTAR ALBINO RATS

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Wound healing, Incision wound model, Excision wound model, *Ziziphus xylopyrus* (Retz) Willd leaves.

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ABSTRACT: Aim: The present study provides a scientific evaluation for the wound healing activity of methanolic extract of *Ziziphus xylopyrus* (Retz) Willd leaves (MEZX). Methods: Incision and excision wound models were used to evaluate the wound healing activity of MEZX on Wistar rats. In each model, animals were divided into four groups of 6 animals each. In both model, Group I was assigned as control (ointment base). Group II and III were treated with 5% w/w and 10% w/w MEZX topical ointment respectively. Group IV was treated with standard nitrofurazone ointment (0.2% w/w). The parameters recorded were percentage of wound contraction, epithelialisation period and tensile strength. Results: It was noted that the effect produced by MEZX ointment showed significant (P < 0.001) healing in all wound models when compared with the control group. All parameters such as wound contraction, epithelialisation period and tensile strength showed significant (P < 0.001) changes when compared with the control. MEZX stimulates wound contraction, decreases epithelialisation period and increases tensile strength. Conclusion: This study suggests that MEZX possesses wound healing activity against Incision and excision wound.

INTRODUCTION: Wounds are inevitable events of life, which arise due to chemical or physical injury or microbial infections ¹. Healing is a multidimensional and complex process initiated in response to injury that re-establishes the integrity and function of injured tissues.



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Healing process can be largely categorized into three phases; inflammatory phase consisting the inflammation and establishment of homeostasis; proliferative phase (consisting of granulation, contraction and epithelialisation) and lastly the remodelling phase, which eventually determines the appearance and strength of the healed area ^{2, 3}. Inappropriate care of the wound may interrupt its healing, causing the area to become infected and consequently resulting in protracted wounds. Several drugs obtained from herbal sources are known to increase the healing of diverse types of wounds ¹.

Herbal medicine has become an essential part of standard healthcare, based on a combination of time privileged traditional usage and current scientific research. Medicinal plants are coming into eminence because of the overuse of conventional drugs such as antibiotics which has resulted in the development of resistance in several infectious organisms. Thus, herbal medicines can be more effective than conventional medicines and they can be administered over longer period of time because of its non-toxic nature ^{4, 5}. Several plant products and natural products ⁶, which are composed of active principles, such as flavonoids ⁷, triterpenoids and alkaloids ⁸ have been reported which stimulate the process of wound healing.

Ziziphus xylopyrus (Retz) Willd (F. Rhamnaceae) is commonly known as Jujab in English ⁹. It is a large trailing shrub or a small tree, fortified with spines, up to 4 m in height. Fruits are globose, 3 celled (rarely 2 or 4-celled), with usually a seed in each cell, very hard and woody. It is found in India, Pakistan and China ¹⁰. As per the ethno medicinal information, different parts of this plant possess several therapeutic properties. The fruit powder with apinch of ginger powder, thrice in a day is beneficial for indigestion and stomach ache 11. It also possesses anthelmintic 12, antimicrobial 13, antidepressant ¹⁴, analgesic and anti-inflammatory activities 15. The leaves contain quercetin and quercetin. The fruits contain tannins, oleanolic acid, l-epicatechin, l-leucocyanidin and 3, 3, 4-tri-O-methyl-ellagic acid. The bark contains tannins, d-7, 3', 4'-trihydroxy flavan-3, 4-diol and oleanolic acid. The stem wood of the plant is reported to alkaloids (xylopyrine-A and triterpenoids and flavonoids 15

Though, in spite of various bioactive phytoconstituents and various medicinal activities attributed to this plant, no research has been carried out to shed light on the role of this plant in wound healing. In the light of the above, the current study was undertaken to investigate the wound healing activity and its effects on percentage of wound contraction, epithelialisation period and tensile strength in incision and excision wound model.

MATERIALS AND METHODS:

Plant material: Ziziphus xylopyrus (Retz) Willd leaves were identified and collected from National

Botanical Research Institute, Lucknow, Uttar Pradesh and were authenticated at The Maharani Lal Kunwari P.G. College, Balrampur, India (Ref. No: MLKPG/2013/125). The leaves were washed, shade dried, pulverized into moderately coarse powder, passed through a 40 mesh sieve and stored in an air tight container for further use.

Extraction: The powder was loaded into soxhlet apparatus and was subjected to extraction with chloroform. The extraction was continued till the defatting had taken place. The marc obtained was subjected to methanol extraction in soxhlet extractor. The extraction was continued for 6-7 days to achieve complete extraction. The methanolic extract was concentrated to dryness using rotary evaporator to get the crude extract. The crude extract were stored in airtight containers and kept refrigerated (2-8°C) until further analysis.

Preliminary phytochemical studies: Phytochemical screening was carried out to identify the presence of flavonoids, triterpenoids, alkaloids, glycosides, steroids, gums and mucilage, proteins, amino acids and tannins in MEZX ¹⁶.

Preparation of formulation and standard used: Simple ointment was prepared from 5% w/w and 10% w/w MEZX by trituration method in a ceramic pestle and mortar using White soft paraffin obtained from S.D. Fine Chem Ltd. (Mumbai, India) ¹⁷. About 5 g and 10 g of crude extract were incorporated into the 95 g and 90 g of simple ointment base B.P. respectively. Simple ointment base was used as the control group and was applied twice per day. MEZX ointment was used twice per day to treat different groups of animals. Nitrofurazone (0.2% w/w) obtained from Rexin Pharmaceutical Pvt. Ltd. (Delhi, India) was used as standard drug for comparing the wound healing activity of MEZX in different animal models and was applied twice per day.

Animals: The experiments were carried out with wistar albino rats weighing 200-250g obtained from the Laboratory Animal Services Division of Central Drug Research Institute, Lucknow, India. Research on experimental animals was conducted in accordance with the internationally accepted principles for laboratory animal use and care (1087/07/ CPCSEA). They were kept in

polypropylene cages (22.5 x 37.5 cm) and were maintained under standard housing conditions (room temperature, 24-27°C and humidity, 60-65 %) with a 12-h light and dark cycle. They were allowed free access to standard pellet diet and water, ad libitium. The experimental protocols were approved by the Institutional Animal Ethics Committee, which follow the guidelines of the Committee for the Purpose of Control Supervision of Experiments on Animals (CPCSEA) and conform to the international norms of the Indian National Science Academy. Ethical norms were strictly followed during all experimental procedures. [Hygia/M.Pharm/28/2012-13]. The rats were anesthetized prior to and during infliction of the experimental wounds.

The surgical interventions were carried out under sterile conditions using ketamine anaesthesia (100 mg/kg b.w., i.p.). Animals were closely observed for any infection and those which showed signs of infection were separated and excluded from the study and were substituted.

Wound healing activity:

Excision wound model: Animals were anesthetized prior to and during making of the wounds. A full thickness excision wound was created for this study according to Morton and Malone. All the animals were shaved on the part to be exposed and ethanol (70%) was applied as antiseptic on shaved region. A round circular seal of 400 mm² diameter was impressed on the dorsal thoracic central region 5 cm away from the ears of anaesthetized rats. Full thickness skin from demarked area was excised to get a wound of approximate 400 mm². After achieving the full homeostasis wound was blotted with cotton swab soaked in warm saline and animals were placed in their individual cages. The wound was left untreated to the open environment and no local or systemic antibacterial agents were applied.

The simple ointment, MEZX and standard were administered topically as per the protocol ¹⁸. All the cages were cleaned on alternative days and the bedding material was changed daily. All the animals were inspected daily and healing was assessed based on physical parameters namely wound contraction and complete epithelialisation period ¹⁹. The animals were

divided into four groups of six rats each. Group I was assigned as control (ointment base). Group II and III were treated with 5% w/w and 10% w/w MEZX ointment respectively. Group IV was treated with standard nitrofurazone ointment (0.2% w/w) once daily from 0 to 21 post wound days or till complete healing, whichever earlier. The wound contraction was assessed by tracing the wound on days 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 post wounding days or till complete wound healing, whichever occur earlier using transparency sheets and a permanent marker. These wound tracings were retraced on 1 mm² graph paper to assess area and then wound contraction was calculated as percentage of original wound size (400 mm²) for each animal in all the groups. Changes in wound area were also calculated to indicate the rate of contraction.

% wound contraction = \frac{\text{wound area on day 0 - wound area on day n}}{\text{wound area on day 0}} x 100

Day n is any specific day on which % wound contraction is calculated ²⁰.

Epithelialisation period: The period of epithelialisation was calculated as the number of days required for falling of the dead tissue fragments of the wound without any residual raw wound ¹⁹.

Incision wound model: In incision wound model, 6 cm long paravertebral incision were made through the full thickness of the skin on either side of the vertebral column of the rats, after all the animals of each group were anesthetized under light ether anaesthesia 21. No systemic or local antimicrobial agents were applied throughout the experiment. All groups were treated same as in excision model, the both edges kept together and stitched with black silk surgical thread (no. 0000) and a curved needle (no. 9) was used for stitching. The continuous threads on both wound edges were tightened for good closure of the wound. After stitching, wound was left undressed then ointment base, MEZX ointment and standard ointment were applied daily until 10 days; when wounds were cured thoroughly the sutures were removed on the day 10 and tensile strength of cured wound skin was measured using tensiometer ²².

The anesthetized animal was secured to the table, and a line was drawn on either side of the wound 3 mm away from the line. This line was gripped using forceps one at each end opposed to each other. One of the forceps was supported firmly, whereas the other was connected to a freely suspended light-weight metal plate. Weight was added slowly and the gradual increase in weight, pulling apart the wound edges. As the wound just opened up, addition of weight was stopped and the weights added was noted as a measure of breaking strength in grams. Three readings were recorded for a given incision wound. The average reading for the group was taken as an individual value of breaking strength. The average value gives the breaking strength for a given group ¹⁹.

Tensile strength = $\frac{\text{breaking strenght (g)}}{\text{cross section area of skin (mm2)}}$

Statistical analysis:

All values are reported as mean \pm S.D. The results were analyzed statistically using one-way analysis of variance (ANOVA). All tests were conducted using Graph Pad Prism Software, Inc. (San Diego, CA).

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RESULTS: In excision wound study, the significant increase in the wound-healing activity was observed in the animals treated with MEZX compared with those who just received the ointment base (control group). Both 5% w/w and 10% w/w MEZX ointment treated animals showed a significant reduction (P < 0.001) in the wound area (**Table 1, Fig. 1**) and epithelialisation period with compare to control group (**Table 2**).

TABLE 1: THE EFFECT OF MEZX ON WOUND CONTRACTION IN EXCISION WOUND MODEL

Parameter	Wound area (mm ²) and Percentage wound contraction			
Post wounding days	Control	5% MEZX	10% MEZX	Standard
Day 2	390.25±1.678	393.25±2.181	375±3.353	348.75±2.966
	(0.15 ± 0.160)	(1.625 ± 1.625)	(7.625 ± 0.344)	$(21.188.\pm0.735)$
Day 4	371.25±2.947	360.00±3.068	340.00 ± 3.195	316.25 ± 2.390
	(5.898 ± 0.476)	(9.825 ± 0.854)	(15.375 ± 0.344)	(22 ± 0.354)
Day 6	292.5 ± 0.744	286.25 ± 0.743	285.00 ± 2.259	265.00 ± 2.080
	(28.125 ± 0.308)	(27.625 ± 1.096)	(30.745 ± 1.009)	(44.89 ± 1.927)
Day 8	234.00±2.924	215.5 ± 2.159^{a2}	206.5 ± 2.062^{b}	197.5±0.734 ^b
	(48.153 ± 0.89)	(56.306 ± 0.65)	(62.563 ± 0.70)	(66.34 ± 1.020)
Day 10	190.00 ± 2.323	175.00±2.187 a2	162.5 ± 2.623^{b}	151.25±2.636 ^b
	(66.1 ± 0.68)	(71.61 ± 0.88)	(76.14 ± 0.57)	(81.83 ± 1.36)
Day 12	153.75±1.093	$123.75\pm1.664^{a2,b2}$	116.25 ± 1.664^{b2}	105.75 ± 1.651^{b}
	(78.35 ± 0.39)	(80.74 ± 1.026)	(84.01 ± 0.70)	(85.6 ± 0.56)
Day 14	109.00±1.450	97.5 ± 0.77144^{a2}	92.25 ± 1.232^{b}	80.75 ± 0.864^{b}
	(81.67 ± 0.44)	(87.25 ± 1.45)	(90.62 ± 1.21)	(92.35 ± 0.54)
Day 16	66.25±1.931	45.00 ± 1.796^{a}	38.75 ± 1.931^{b}	24.5 ± 1.025^{b}
	(91.67 ± 0.409)	(93.78 ± 0.31)	(94.25 ± 0.91)	(95.615 ± 0.602)
Day 18	53.75 ± 1.369	22.5 ± 1.270^{a2}	15.00 ± 1.010^{b}	11.25 ± 0.612^{b}
	(95.30 ± 0.13)	(96.89 ± 0.28)	(97.47 ± 0.46)	(99.36 ± 0.094)
Day 20	12.75±1.232	6.25 ± 0.811^{a}	5.25 ± 0.693^{b}	1.00 ± 0.5
	(97.18 ± 0.255)	(97.675 ± 0.369)	(99.127 ± 0.181)	(100 ± 0.00)

Values were expressed as mean \pm S.D, (Figures in the parenthesis indicate % wound contraction). ${}^{a}P < 0.0001$, ${}^{a^{2}}P < 0.001$, ${}^{a^{3}}P < 0.01$ when compared to control group. ${}^{b}P < 0.0001$, ${}^{b^{2}}P < 0.001$, when compared to standard group.

TABLE 2: THE EFFECTS OF MEZX ON PERIOD OF EPITHELIALISATION IN EXCISION WOUND MODEL.

Group	Treatment	Epithelialisation Period (mean time in days)
I	Control	26.5±0.813
II	5% MEZX	22.75±0.791** ^a
III	10% MEZX	19.75±0.267*** ^b
IV	Standard	18.25±0.165***

Values were expressed as mean \pm S.D. **P <0.01, ***P <0.001, a- indicates comparison with control and b- indicates comparison with standard group.



FIG. 1: PHOTOGRAPHIC REPRESENTATION OF CONTRACTION RATE SHOWING PERCENT WOUND CONTRACTION AREA ON DIFFERENT POST EXCISION DAYS OF CONTROL, MEZX (5% W/W AND 10% W/W) AND STANDARD NITROFURAZONE OINTMENT (0.2% W/W) TREATED RATS.

Table 3 depicts the wound-healing effect of 5% w/w and 10% w/w MEZX ointment in the incision wound model. In incision wound study, a significant increase (P < 0.001) in the wound-

breaking strength was observed with the 10% w/w MEZX ointment when compared with the control group.

TABLE 3: THE EFFECT OF MEZX ON WOUND TENSILE STRENGTH IN INCISION WOUND MODEL

Group	Treatment	Tensile strength in (g/mm²) 10 th day
I	Control	63.8±0.805
II	5% MEZX	$76.25 \pm 1.665^{**a}$
III	10% MEZX	110±2.324***b
IV	Standard	152.5±2.5***

Values were expressed as mean \pm S.D. **P < 0.01, ***P < 0.001, a- indicates comparison with control and b- indicates comparison with standard group.

DISCUSSION: Wound healing is a process by which an injured tissue gets re-established as closely as possible to its normal state and wound contraction is the progression of shrinkage of area

of the wound. It mainly depends on the repairing capability of the tissue, type and extent of injury and general state of the health of the tissue. The granulation tissue of the wound is primarily constituted of collagen, fibroblast, edema and small blood vessels. The new homogeneous mesenchymal cells of the wound margin modulate themselves into fibroblast, which start wandering into the wound gap along with the fibrin strands. composed The collagen of amino (hydroxyproline), which is the chief component of extra cellular tissue, which gives support and strength ²³. Preliminary phytochemical screening exposed that MEZX showed positive response to flavonoids, triterpenoids, alkaloids, gums and mucilage, amino acids, tannins, glycosides and steroids. Flavonoids are known to reduce lipid peroxidation not only by preventing or slowing the onset of cell necrosis but also by improving vascularity. Hence, any drug that inhibits lipid peroxidation is believed to increase the viability of collagen fibrils by increasing the strength of fibres, increasing the circulation, collagen preventing the cell damage and by promoting the DNA synthesis ²⁴. Flavonoids ²⁵, triterpenoids ²⁶ are also known to promote the wound-healing process mainly due to their astringent and antimicrobial property, which seems responsible for wound contraction and increased rate of epithelialisation.

CONCLUSION: In summary, this study highlights the potential wound healing activity of *Ziziphus xylopyrus* (Retz) Willd leaves. Hence, the wound healing activity of *Ziziphus xylopyrus* (Retz) Willd leaves may be attributed to the phytoconstituents present in it, which may be either due to their individual or the synergistic effect that fastens the process of wound healing. Further phytochemical studies are needed to isolate the active constituent(s) responsible wound healing activity.

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authors alone are responsible for the content of the paper.

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