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Research Article

EVALUATION OF THE WOUND HEALING ACTIVITY OF THE ETHANOLIC EXTRACT OF THE LEAVES OF *MELIA AZEDARACH*

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Abstract
Being a rich heritage of medicinal plants in India there is always a hope for the valued medicinal plants for curing some of the untreated ailments. For the treatment of wounds no. of medicinal plants has been studied since yet. In the continuation of the same the present study is a step towards the healing of wounds by using the leaves of *Melia azedarach*. The efficacy of ethanolic extract of leaves of *Melia azedarach* evaluated in excision and incision wounds models and it was found that the extract showed marked effect in wound contraction, epithelization time in excision wound model and tensile strength in incision wound model with 5 % and 10% ointment of plant extract for 16 days. Povidone-iodine ointment (10%) was used as a standard and Student's t test was used for analyzing the data obtained from the study and the value of $P < 0.05$ and $P<0.001$ was considered to be significant. The effects obtained from ethanolic extract of *Melia azedarach* was compared with control and it was found significant.

Keywords: - : *Melia azedarach*, Excision wound model, Incision wounds model, epithelization time. Ethanolic extract. Povidone-iodine ointment.

Introduction

Wound is the injury occurs due to the opening or removal of epidermis or dermis by any kind of accident and wound healing is the process of regeneration of dermis and epidermis tissue. Loss of epidermis only called superficial wound if both the epidermis and dermis involves then it is called partial thickness wound and if the dermis, subcutaneous fat and sometimes bone also involves then it is called full thickness wound. The wound healing process is a dynamic one which can be divided into three phases. Inflammatory phase then proliferation phase and in the last maturation phase. For the healing of wound here the leaves of *Melia azedarach* has choosen. *Melia azedarach* is a species of deciduous tree in the mahogany family, Meliaceae, that is native to India, southern China and Australia. Common names include Persian Lilac, White Cedar, Chinaberry, Bead Tree . It has variety of uses such as antifungal (Jabeen K., *et al*., 2010), potent insecticidal (Xu H., *et al.*,2010), anti-inflammatory (Al-Badrani *et al*.,2002), analgesic (Vishnukanta et al., 2010), hepatoprotective (Rao *et al.*,2012),viral infections(Barquero *et al.*,1997), antiulcer (Bahuguna *et al.*,2009),stress-induced ulcers (Hanifa et al.,1984), lymphocytic lukemia (Itokawa *et al*.,1995).The extract of leaves of *Melia azedarach*, which contains highest amount of phenolic compounds, exhibited the greatest anti-oxidant activity (Ahmed *et al.*,2012**)**.The present study was undertaken to study the wound healing effect of ethanolic extract of leaves of *Melia azedarach.*

Materials & Methods

Plant material:

Fresh and young green leaves of *Melia azedarach* were collected from the hisar agriculture university, Hisar, Haryana and got identified and authenticated by Dr. H.B. Singh Scientist G & Head, Raw Material Herbarium and museum (RHMD) with voucher specimen no. (NISCAIR/RHMD/Consult/2011-12/1766/66) and the specimen was kept in National Institute of Science Communication And Information Resources (CSIR-NISCAIR), Raw Material Herbarium and Museum, New Delhi.

Extraction:

The collected leaves were dried in shade, pulverized by a mechanical grinder to get the coarse powder. The dried coarse powder then extracted with ethanol at ambient temperature, filtered and evaporated to dryness under reduced pressure in the Rotary Evaporator (Bhuchi type) at 40–45 ^{0}C.The extract were kept in amber colored bottles and kept in a desiccator. The part of the extracts was studied for wound healing activity**.**

Experimental animals :

Healthy male albino rats of wistar stain weighting b/w 150-250 gm were selected for the study and housed and maintained (23± 4 ^{0}C, relative humidity 60–70%) in the animal house of Department of Pharmacy, Bundelkhand University, Jhansi and kept on a standard diet with water ad libitum and were acclimatized for two weeks before the experimentations. The protocol of the study was approved by the concerned ethical committee for animal experimentation with no. (Bu/Pharm/IACE/11/036). Animals were periodically weighed before and after the experiment. The rats were anesthetized prior to and during infliction of the experimental wounds. The surgical interventions were carried out under sterile conditions using ketamine hydrochloride anesthesia (100 mg/kg, 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 replaced.

Evaluation of wound-healing activity

Excision and incision wound models were used to evaluate the wound-healing activity of ethanolic extract of leaves of *Melia azedarach*.

Excision wound model:

The rats were anaesthetized prior to and during the creation of wounds with100mg/kg of ketamine hydrochloride. The dorsal fur of each animal was shaved with electric clipper and then inflicted with excision wound (500mm^2) along the marking by a sterile toothed forceps, a surgical blade and a pointed scissor (Nayak et al.,2009), and the entire wound was left open (Morton and Malone, 1972). The rats includes in study was divided in four groups having five rats in each group. Group I animals treated topically with the water soluble ointment base and serves as control. Group II animals treated topically with 5% ointment of ethanolic extract of leaves of *Melia azedarach*. Group III animals treated topically with 10 % ointment of ethanolic extract of *Melia azedarach*. Group IV animals treated topically by povidone-iodine ointment (10%) till complete epithelialization. The curing of wound was assessed by tracing the wound on 4,8,12 and 16th days using transparency paper and a marker, and the recorded wound areas were measured graphically. Number of days required for falling of eschar was calculated as period of epithelialization (Morton and Malone,1972; Nayak *et.,al*.,2009).

Incision wound model:

A 5 cm long abdominal incision was make in shaved area of anaesthetized rat and closed with surgical thread (No.000) and curved needle (No.11)at a distance of 1 cm. There after they were kept individually in different cages **(**Ehrlich and Hunt, 1969).

Measurement of Tensile strength :

Treatment was given to animals as describe above for 14 days. Sutures were removed on 12th day and treatment was continued up to 14th day. On the 14th day the animals were sacrificed by cervical dislocation and there tensile strength of recovered area was measured. Wound areas from each animal were removed carefully. Wound stripes of equal size and width were then cut using a knife in which two blades(No. 23) were fixed at a fixed distance. Both ends of each strip were fixed with the help of a pair of steel clips. One clip allowed hanging on

a stand and a polyethylene bag was then allowed to fill with water gradually 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 (Koback, 1965).

Toxicity study

Oral Toxicity

The acute toxic class method was a stepwise procedure with the use of 3 animals of a single sex per step. Acute oral toxicity was performed as per OECD-423 guidelines. The animals were fasted overnight with water *ad libitum*. The starting dose of 5 mg/kg of ethanolic extract was administered orally to three animals in each group. If mortality was observed in two or three animals, then the dose administered was assigned as a toxic dose. If mortality was observed in one animal, then the same dose was repeated again in three animals to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such as 50, 300, and 2000 mg/kg body weight. Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total of 14 days.

Dermal Toxicity

Toxicity of extract was also checked topically as per OECD 410,which is of 21 days study on at least ten animal (5 female and 5 male of at least 200 gm acclimatised at standard condition).Selected dose(1000 mg/kg body weight) should be applied on shaved area (24 hr before and repeated weekly)on at least 10 % area ideally for at least 6 hours per day on a 7-day per week basis, for a period of 21days to control and treatment groups and observed daily for any signs of toxicity. If any animal died due to high dose should separate out from study.

Statistical analysis

The results obtained were expressed in mean ± SE. The statistical significance was evaluated by student's t test to compare the differences between experimental groups with control using $P<0.001$ and $P<0.05$ was considered statistically significant.

Table 1 : The wound healing effect of ethanolic extract of leaves of *Melia azedarach* on wound contraction in open wound of normal rats in excision wound model.

Gp	Treatments	Wound area (mm^2)					Epithelization time
		Day 0	Day 4	Day 8	Day12	Day 16	
I	Control (water soluble ointment base)	503±0.58	416±1.68	322±3.30	196±2.13	78±1.23	20±0.56
II	*Melia azedarach (EE) (5% ointment)*	502±0.51	365.6±2.13*	237.2±2.05*	123.2±3.2*	55±2.11*	18.8±0.64*
III	*Melia azedarach (EE) (10% ointment)*	504±0.71	310±1.84*	210.2±1.55*	81.4±2.70*	24±2.32*	17.2±0.46*
IV	Povidone Iodine ointment (10%)	502±0.51	293.2±3.25*	185.4±2.13*	62.8±2.05*	00±00*	15.4±0.52*

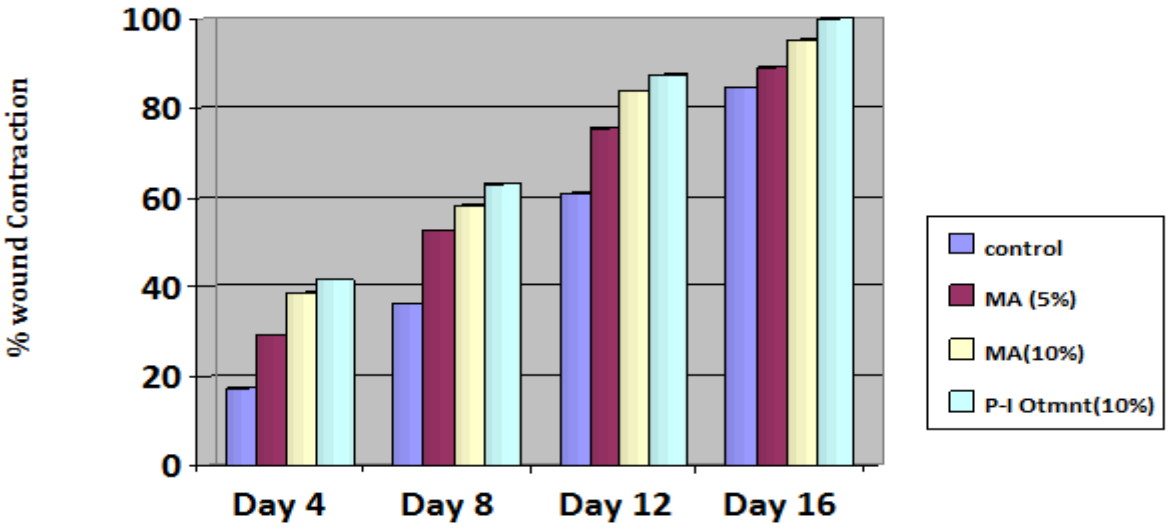
Values are mentioned above are as mean ±SE, (n = 5). * P<0.001verses control considered to be significant followed by student's t test. ** P<0.05 verses control followed by student's t test.

Table 2 The wound healing effect of ethanolic extract of leaves of *Melia azedarach* on tensile strength in incised wound of normal rats in incision wound model.

Gp	Treatments	Tensile strength (gm) on 14th day of treatment
I	Control (water soluble ointment base)	245.18±6.35
II	*Melia azedarach (EE)* (5%)	325.60±5.12*
III	*Melia azedarach (EE) (10%)*	400.5±4.21*
IV	Povidone Iodine ointment (10%)	425.94±7.19*

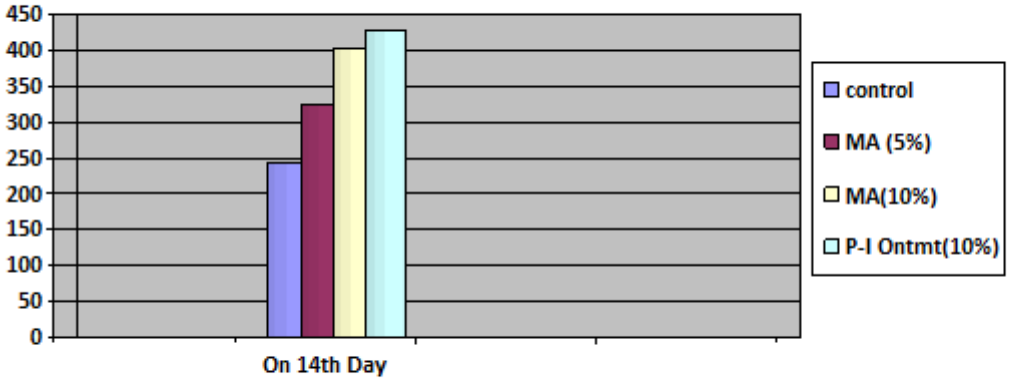
Values are mentioned above are as mean ±SE, (n = 5).* P<0.001 verses control followed by student`s t test. ** P<0.05 verses control followed by student`s t test.

Figure 1 Percentage wound contraction of ethanolic extract of leaves of *Melia azedarach* on excision wound in alloxan induced diabetic rats.



The wound contraction found significant in both of the concentration of the extract i.e. Mekia azedarach 5% and Melia azedarach 10% in the water soluble ointment base.

Figure 2 The wound healing effect of ethanolic extract of leaves of *Melia azedarach* on tensile strength in incised wound of normal rats in incision wound model.



The above graph shows the tensile strength in incised wound of normal rats in incision wound model Here amount of water used in gms on 14th day in different groups shows the effect in control, Melia adedarach 5%, Melia adedarach 10% and povidone Iodine ointment 10%.

Result & Discussion

The Ethanolic extract of the leaves of *Melia azedarach* in different concentration i.e. 5% and 10% showed significant effect for decreasing the wound area and the epithelization time in the excision wound and showed marked effect in tensile strength in the incision wound model. *Melia azedarach* 5% ointment showed epithelization time in 18.8 days.Whereas *Melia azedarach* 10% showed epithelization time in 17.2 days. Control showed epithelization time in 20 days Povidone Iodine ointment (10%) showed epithelization time 15.4 days. So the ethanolic extract of leaves of *Melia azedarach* is effective for wound healing.

Toxicity test

There was no sign of toxicity were observed in oral and dermal toxicity studies in the given dose for the prescribed period of time.

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