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Evaluation of Wound Healing Potential of Seeds and Leaves of *Cordia Obliqua* Wild

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***Reenu Yadav[1], Ankur Chaubey[2], Akshat Sharma[3], Abhay Asthana[4], Prashanti Ch [5]**

[1]IES College of Pharmacy, Bhopal (M.P.),[2]School of Pharmacy, Suresh Gyan Vihar university, Jaipur , [3]LNCP, Bhopal (M.P.), [4]MMCP, MM University Mullana, (H.R.), [5]Bagdevi College of Pharmacy, Warangal (A.P.)

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ABSTRACT

Objective of this present work is to evaluate wound healing potential of the aqueous and methanolic extract of the seeds and leaves of *Cordia obliuqa*. Three models (incision, excision and dead space wound) were used to evaluate healing activity of the extracts. Wounds are created to the rats (albino rats), in excision model both the extracts were applied till epithelialization process completed. On other hand in incision and dead space wound model extracts were applied daily (topically) till 10th day after wound formation. While standard group was treated with povidone iodine ointment topically. Observation was carried out for percentage of wound contraction, breaking strength, epithelialization period, hydroxyproline content and granulation weight. Both the extracts provided significant results ($p<0.05$) as compared to the standard control group. Methanolic extract is more effective than the aqueous extract, but both the extracts were able to decrease epithelialization period, increase percentage wound contraction in excision model. In dead space model both the extracts increased hydroxyl proline content, and also increased breaking strength in incision model.

INTRODUCTION

The Boraginaceae family comprises about 2740 species distributed in 148 genera [1]. The genus Cordia is one of the most representatives of this family and the chemical characteristic in this genus is the presence of quinones known as cordiaquinones. However, flavonoids, terpenoids, carbohydrates, lipids and phenylpropanoids are also well reported for the genus [2]. Cordia is a genus of trees or shrubs, sometimes subscandent in the borage family Boraginaceae. About 300 species have been identified worldwide, mostly in warmer regions. The plant parts like fruits, leaves, stem bark, seeds and roots of most species of plants of the genus Cordia, especially *Cordia dichotoma, C. myxa, C. obliqua, C. verbenacea, C. martinicensis, C. salicifolia, C. spinescens, C.latifolia, C. ulmifolia*, among others, has long been used in traditional medicine for cicatrizant, astringent, anti-inflammatory, anthelmintic, antimalarial, diuretic, febrifuge, appetite suppressant, cough suppressant and to treat urinary infections, lung diseases and leprosy [3-5]. *Cordia obliqua* commonly known as Lasora. It is a medium sized tree. *Cordia obliqua* is native to mid Himalayan region, and found upto elevations of 1470 meters. *Cordia obliqua*, found in two forms and these two forms can be differentiated by the size of the fruits. One form consist larger fruits and other one has small size fruits. It is useful as an expectorant and effective in treating the diseases of the lungs. In the raw condition, they contain a gum which can be used beneficially in gonorrhoea [6]. Seeds are utilized as an anti-inflammatory agent [7]. The fruits are also useful in treating coughs, the diseases of the chest, and chronic fever. They lessen thirst and the scalding of the urine, remove pain from the joints and the burning of throat and are also effective in treating the diseases of the spleen. Fruits are used as a demulcent in southern Iran [8]. Root of the plant contains Hesperetin 7- rhamnoside**,** a glycoside of hesperetin Lupa-20, 29-ene-3-o-D-maltosideand [9,10,11]. Toxifolin-3, 5-dirhamnoside isolated from the seeds of the plant [12]. Fruits contain natural gums and mucilages [13].

A wound can be defined as it is a break in the integrity of epithelial tissues of the skin. It can break the cellular and anatomical function of the living tissues of the skin [14]. Wound healing may be defined as a process by which skin repairs it after getting an injury [15]. When an injury occurs to the skin, a set of complex biochemical events takes place repair the damage. After injury, within minutes platelets (thrombocytes) aggregate at the injury site and form a fibrin clot. This fibrin clot controls active bleeding (hemostasis). Wound healing speed can be impacted by many factors, which includes the bloodstream levels of hormones (oxytocin) [17]. We study here wound healing process, to discuss various internal and external factors which can affect the healing process and could use in clinical practice for better healing or in the alteration of healing process favorably. Wound healing process takes place in various continuous phases. 1. Hemostasis 2. Inflammatory 3. Proliferative 4. Remodeling. [16].

Wound healing process summarized as coagulation, inflammation, remodeling of connective tissues, collagenization and possessing strength to the wound. When a new tissue forms, endothelial proliferate and they form new blood vessels [18]. Many plants are reported to have wound healing potential such as *Aloe vera, Tridex procumbens, Leucas lavandulaefolia* [19] *Aspila africana* [20], *Cassia tora, Carapa guianensis, Alternanthera sessilis* L, *Allum Cepa* L, *Mussaenda frondosa*. L, *Moringa oleifera* L, *Lantana camara* L., Ageratum conyzoides *Heliotropium Indicum* [21] Tecomaria capensis [22] *Mimosa pudica* [23] Carapa guianensis [24] *Centella asiatica* [25].

MATERIALS AND METHODS

Plant material

Fresh leaves and seeds of *C. obliqua* wild were collected from forest area of adjoining to Dist. Chhatarpur (M.P) in the month of April and authentication of plant material (specimen voucher no. 864/22) was done by taxonomist Dr. Manjusa Saxena at Department of botany, Govt. Maharaja College, Chhatarpur (M.P.).

Preparation of extracts

Dried and powdered seeds and leaves of *Cordia obliuqa* (250 gm) defatted firstly to remove fatty materials from the extract. Weighed powdered material is packed with petroleum ether (500 ml) at 30-40^{o} C for 72 hours in Soxhlet apparatus. And extraction was confirmed to ensure by pouring a drop of extract from the thimble on a filter paper, which does not show the presence of any oil spot on that (Intermittent filter paper stain test is done to ensure complete defatting). After completion of extraction, last traces of petroleum ether defatted extract material was removed from the Soxhlet and air dried. Defatted plant material was again subjected to hot continuous extraction with (500 ml) 95% methanol (30-40^{o}C) in a Soxhlet apparatus for 24 hours, and aqueous extract were prepared by maceration technique of extraction. Extracts were concentrated on water bath and powdered.

Phytochemical analysis

Phytochemical studies were performed on the basis of literature available. [26, 27]. Several phytochemical tests were performed for testing various different chemical groups present in methanolic and aqueous extracts.

High Performance Thin Layer Chromatography

HPTLC Plates 3 cm x 10 cm, coated with 0.25 mm layer of silica gel 60 F254 (Merck, Germany) was used for the chromatography. Plates were washed with methanol and activation was done at 110^{O}C for 5 min. Samples were applied using a Camag (Muttenz, Switzer land) Linomat IV applicator equipped with 100 µL syringer. Samples were applied as 4 mm wide bands and 6 mm apart, a constant application rate of 5 µL/s was used.

Animals

Study was carried out in the laboratory of VNS Institute of pharmacy Bhopal (M.P.). This study was approved by institutional ethics committee, VNS institute of pharmacy, Bhopal for animal experimentation. All the experiments and its procedures were performed as per CPCSEA guidelines, India. Wister albino rats of either sex (150 -200 g) were utilised for the study. Rats were stabilized for one week and they were maintained in standard condition at room temperature, relative humidity (60± 5%) and 12 h light and dark cycle. They were provided standard feeds according to the guidelines. Oral route of administration was chosen for all extracts.

Excision wound model

Approximately 500 mm thick, circular piece from dorsal area of the rat was removed and a wound is created. The wound was traced on the day of wounding. It was traced daily till the complete healing occurs on 1 mm graph paper. Rate of wound contraction calculated on the basis of changes occurred in the wound size. Period of epithelialisation is considered as the number of days required for falling the eschar without any residual of raw wound [18,28].

Incision wound model

Anaesthesia (Ketamine 0.5 mL/kg bw. i.p.) was given to the rats. Anaesthetized rats were shaved back (after 30 min. of administration of ketamine injection) and incision wound were made (6 cm in length and 2 mm in depth) with the help of sterile scalpel. A tighten stitching was done of parted skin by keeping them together with the help of black silk surgical thread (No. 000) and needle (No. 9). Wounding day was considered as day 0. Rats of different groups then treated with extracts for treatment of their wounds by oral administration. Sutures were removed on 8^{th} day and tensile strength of the skin was measured by tensiometer on 10^{th} day of creating wounds [18].

Dead space wound model

A transverse incision in the lumber region on either side of vertebral column of the rats was made to create dead space wounds. And two polypropylene tubes were inserted (subcutaneously) on the either side of the vertebral column to harvest the granulation tissue. Wounding day was considered as 0 day and rats were treated from the extract from 0 day to 9^{th} post wounding day. Granulated tissue dissected carefully on 10^{th} post wounding day, and continuous and constant water flow technique was utilized to measure tensile strength of the skin [18, 28, 29].

Hydroxyproline estimation

The dissected tissue was dried in oven (at $60^{O}C$) for 24 hours and dry weight was noted. Dried tissue were hydrolysed in a sealed glass tube in 6 N HCI for 4 hours at $130^{o}C$, then neutralized to pH 7 and subjected to Choramine-T oxidation for 20 min. After addition of 0.4 M perchloric acid, color was developed with the help of Ehrlich reagent at $60^{o}C$. Spectrophotometer (Shimadzu 1700, Japan) was used for the measurement of the absorbance.

RESULTS

Results from phytochemical analysis showed presence of flavonoids, phenolics, alkaloids tannins and saponins. On other hand amino acids and carbohydrates are found absent. Further presence of these chemical constituents was confirmed by HPTLC analysis.

Extract treated group showed an increase in the breaking strength of the incision wounds significantly, results were comparable to the standard drug povidone iodine and found significant ($p<0.05$).

The wound contraction ability of the extracts (aqueous and methanolic) of *Cordia obliqua* was significantly greater ($p < 0.05$) than that of control. Aqueous extract showed greater wound contraction and healing activity in comparison to methanolic extract and standard drug. The wound closure time was lesser and percentage healing was greater (14 days for 100 % healing).

Table 2 showed that both the extracts of *Cordia obliqua* shown significant increase in granuloma breaking strength as compared to standard drug. Significant increase in hydroxyproline content was observed by both the extract treated group as well as by the standard drug ($p<0.05$).

Table 1. Percentage of wound contraction and period of epithelialization in excision wound model

Groups	Percentage wound contraction					Period of epithelialization
	Day 2	Day 4	Day 8	Day 12	Day 16	
Povidone iodine	10.50±0.23	43.83±0.54	73.44±0.33	84±0.45	92±0.31	16.47 ±0.9*
Aqueous extract of *Cordia obliuqa*	14.42±0.4	66.00±0.5	84.78±0.45	99.00±0.2	100.32±0.12	13.31±0.68*
Methanolic extract of *Cordia obliqua*	13.55±0.32	55.88±0.33	73.32±0.66	89.44±0.76	94.48±0.38	14.90±0.22*
control	3.25±0.44	13.55±0.44	43.55±0.54	70.14±0.22	78.12 ±0.12	18.86±0.23

* $P<0.05$ when compared with standard group

Table 2. Hydroxiproline content, granulation breaking strength and dry granulation weight on 10th day of dead space model

Groups	Hydroxyproline content (mg/ml)	Granulation breaking strength	Dry granulation weight (mg/g)
Standard povidone iodine	34.20±.0.2*	78.20±1.14*	42.24±1.12*
Aqueous extract of *Cordia obliuqa*	51.10±.0.30*	98.56±1.44*	58.26±1.02*
Methanolic extract of *Cordia obliqua*	46.20±2.00*	88.78±2.14*	48.20±0.14*
Control	25.40± 2.22	59.40± 2.10	32.40± 1.22

* P<0.05 when compared with standard group

DISCUSSION

The present investigation describes some unique features of the seeds and leaves extract from the plant *Codia obliqua* with respect to its potential wound healing capacity in rats. Plant products are potential wound healing agents, and largely preferred because of their widespread availability, non-toxicity, absence of unwanted side effects, and effectiveness as crude preparations.

The phytochemical screening results reveal the presence of tannins, alkaloids, flavonoids, phenolics and saponins in the extracts. The constituents of the extracts, such as terpenoids and alkaloids, may play a major role in the wound healing process which was observed in the study; however, further phytochemical studies are needed to isolate the active compound(s) responsible for these pharmacological activities.

The breaking strength of wounds was increased in aqueous and methanolic extract treated groups.

Depositions of newly synthesized collagens were increased at the site of wound. Collagens concentration per unit area was increased; hence the tissue tensile strength was increased.

Significant increase in percentage closure by enhanced epithelialization was showed by aqueous and methanolic extracts of *Cordia obliqua* in excision wound healing model. This increased epithelialization may be due to the effect of *Cordia obliqua* on enhanced collagen synthesis.

Observations from the study of dead space model showed both the extract treated groups increased granuloma tissue breaking strength, which is an indication of better healing activity.

According to the experimental results, crude aqueous extract of *Cordia obliqua* showed better wound healing activity compared to methanolic extract and even compared to standard povidone iodine ointment.

CONCLUSION

The results obtained in the present study clearly indicate that the extracts of *Cordia obliqua* are having significant wound healing activity in rats. The wound healing effect of extracts of *Cordia obliqua* may be due to the presence of more than one active principles mentioned above. Further pharmacological and biochemical investigation will clearly elucidate the mechanism of action and will be helpful in projecting this plant as a therapeutic target in wound healing as well as other diseases. Development and evaluation of a formulation including these extracts are the future challenges.

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