Wound healing activity of *Cordia dichotoma* Forst. f. fruits

I J Kuppast[1]* and P Vasudeva Nayak[2]

[1] Department of Pharmacology, National College of Pharmacy
Balraj Urs Road, Shimoga-577 201, Karnataka, India
[2] Department of Studies in Chemistry, Kuvempu University, Jnana Sahydri
Shankarghatta-577 451, Karnataka, India
*Correspondent author, E-mail: ijkuppast@yahoo.com

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Abstract

The extraction of fruits of ***Cordia dichotoma* Forst. f.** was carried out using ethanol. This extract was further fractionated using petroleum ether (40-60%), solvent ether, ethyl acetate, butanol and butanone in succession. These fractions were screened for wound healing activity using three different models, viz. excision, incision and dead space wound models on either sex of albino rats of Wistar strain. All the fractions showed significant ($P<0.001$) activity on the chosen models.

Keywords : *Cordia dichotoma*, Ethanol extract, Flavonoids, Wound healing activity.

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Introduction

Cordia dichotoma* Forst. f.** (Hindi — *Lasora*) belonging to family *Boraginaceae* is a medium sized tree with a short, usually crooked trunk (90-120 cm in girth)[1] and bearing globose, yellowish-brown, pink or black and pulpy fruits, grows in India, Sri Lanka and other warmer countries. The medicinal attributes of ***C. dichotoma have been known since long time. Its fruits are used as cooling, astringent, emollient, expectorant, anthelmintic and purgative[2]. Analgesic, antiinflammatory and hepatoprotective activities have also been reported from the plant[3-5]. The fruits contain large quantities of amino acids, flavonoids, and saponins and are used as wound-healing agent in households. The detailed investigations of this aspect has not been carried out so far, which led us to study the wound healing property by excision; incision and dead space wound models.

Materials and Methods

The fruits (5kg) of ***C. dichotoma*** purchased from local market of Hubli were authenticated (Department of Botany, H.S.K. Science and S.K. Arts College, Hubli), shade dried and prepared a coarse powder. The fruit powder was subjected to repeated soxhlation in batches with ethanol. After complete extraction the alcoholic extract was suspended and further fractionated using petroleum ether (40-60%), solvent ether, ethyl acetate, butanol and butanone in succession. The percentage yield of fractions were 15, 8, 12, 7.5 and 5.2g for petroleum ether, solvent ether, ethyl acetate, butanol and butanone, respectively. These fractions were vacuum dried and used to study wound healing property for excision wound models. Tween-80 (1%) was used as vehicle to suspend the fraction concentrates for incision wound model p.o.

The ethanol extract and ethyl acetate and butanol fractions of fruits showed positive test for flavonoids. The presence of flavonoids were analysed by Shinodha and Zn-HCl reduction test and confirmed by TLC studies using Silica Gel-G as stationary phase and combination of chloroform : acetone : formic acid (70:20:10) as mobile phase. After activation the flavonoid spots were identified by spraying anisaldehyde sulphuric acid. For further study the flavonoids were isolated by column chromatography technique. Finally the isolated pure compounds were subjected to spectral analysis for structural elucidation.

*Pharmacological screening**

Acute toxicity study[6]

Albino rats of either sex weighing 150-200 g were used to determine the dose. The animals were fasted overnight prior to the acute toxicity study where the method of "Up and Down" was used for the dose determination. Tween-80 (1%)

was used as a vehicle to suspend the fractions and was administered orally.

Wound healing activity

Albino rats of either sex of Wistar strain weighing 150-200g procured from animal house of K.L.E.S' College of Pharmacy, Vidyanagar, Hubli were housed in polypropylene cages with paddy house bedding under standard laboratory conditions. Food in the form of dry pellets and water was made available *ad libitum*. The basal food intake and bodyweight to the nearest gram were noted. Under light ether anaesthesia wounding was performed aseptically.

Three models in albino rats i.e. (i) excision wound, (ii) incision wound and (iii) dead space wound, were used for assessing the wound healing activity. The rats were anaesthetized prior to excision and other surgical procedures and finally were sacrificed by exposing to higher dose of anaesthetic ether prior to determination of the tensile strengths of the resutured wounds and the removal of granuloma tissues (grass pith).

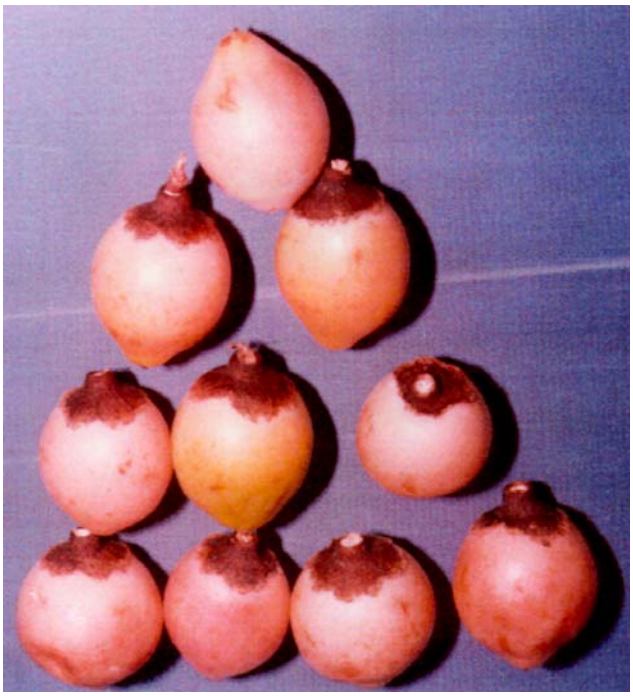
The approval of the Institutional Animal Ethical Committee was taken for study.

(i) Excision wound

For the excision wound study[7], each group containing six animals were used. A circular wound of about 2.5 cm diam. was made on depilated dorsal thoracic region of rats under light ether anaesthesia in aseptic condition and observed through out the study. The animals were housed individually. The suspensions of extracts under study prepared in 1% Tween-80 were administered daily for 14 days starting from the day of wounding. The observations for wound closure were made on 4th, 8th, 12th and 14th post wounding days.

(ii) Incision wound

The method of Ehrlich and Hunt[8] was used where under light ether anaesthesia rats were wounded and two-paravertebral incisions of 6 cm were made through the entire thickness of the skin, on either side of the vertebral column with the help of a sharp blade. The incisions were sutured using 4-0 silk thread with the help of straight rounded bodied needle. The sutures were removed on 8th post wounding day and the tensile strength was determined on 10th post wounding day by continuous constant water flow technique.



Cordia dichotoma fruits

(iii) Dead space wounds (Granuloma studies)[8]

Physical changes in the granuloma tissues were observed under this study. Under light ether anaesthesia, in rats subcutaneous dead space wound were inflicted in the region of the axilla and groin, by making a pouch through a small nick in the skin. Implanting grass pith induced the formation of tissues on the pith. Cylindrical grass piths measuring 2.5 cm in length and 0.3 cm in diam. were introduced into the pouch. Each animal received 2 grass piths in different locations.

The wounds were sutured and mopped with an alcoholic swab. Animals were placed into their individual cages after recovery from anaesthesia. Excision of the granulomas from the surrounding tissues was performed on the 10th post wounding day under light ether anaesthesia.

Granuloma surrounding the grass piths were excised and slit open. The tensile strength of piece measuring about 15 mm in length and 8 mm in width (obtained by trimming the rectangular strip of granuloma tissue) was determined on 10th post wounding day adopting continuous water flow technique of Lee[9].

Statistical analysis

All the results are reported as mean ±S.E and significance of the difference

Table 1 : Mean percentage closure of excised wound area in sq mm ± S.E. following post wounding days by *Cordia dichotoma* ethanol extracts and fractions

Extract/Fraction	4th day	8th day	10th day	12th day	14th day
Control	27.85±1.16	52.59±1.62	62.81±0.35	78.56±0.71	87.76±0.66
Ethanol	37.45±2.28	67.67±1.16	78.23±0.23	85.36±0.36	90.36±0.86
Pet. ether	44.83±2.60	74.98±1.25	88.58±0.57	98.63±0.26	99.52±0.37
Solvent ether	61.21±0.61	71.84±6.82	84.33±2.42	98.05±0.87	99.72±0.10
Ethyl acetate	51.38±0.88	66.97±1.44	83.90±1.84	95.4±0.53	99.24±0.19
Butanol	62.33±0.69	81.25±52.87	92.39±0.49	99.7±0.11	99.92±0.05
Butanone	46.21±3.08	52.87±1.47	71.92±0.98	89.05±0.21	92.22±0.63

between "control" and "drug treated" groups was determined by the student "t" test.

Results

The doses of the fractions determined by "Up and Down" method, were found to be 300 mg/kg body weight. The results were analysed by student "t" test at a significance level of $P<0.001$. The result of excision wound on 4th, 8th, 12th and 14th days are summarised in Table 1. Contraction of excision wound was promoted from 4th day of treatment till 14th day. In case of rats treated with fractions of ethanol extract of fruits the epithelization of wound was found to be quite earlier as compared to control. The results of incision wound model and that of granuloma studies are reported in Tables 2 and 3, respectively. A significant increase in the tensile strength of test as compared to control suggests that the extracts promote wound-healing activity. Results of excision, incision and granuloma wound models showed significant wound healing property of the fruit extracts of ***C. dichotoma***.

Table 2 : Mean tensile strength (grams) of resutured incision wound on 10th post wounding day by *Cordia dichotoma* ethanol extract and fractions

Extract/Fraction	Dose mg/kg body wt	Grams ±S.E.
Control	-	240 ± 15.27
Ethanol	300	545.36±1.16
Pet. ether	300	650 ± 6.92
Solvent ether	300	648 ± 8.41
Ethyl acetate	300	639 ± 17.94
Butanol	300	660 ± 5.81
Butanone	300	533 ± 8.11

Table 3 : Mean granuloma tensile strengths (grams) on post wounding day by *Cordia dichotoma* ethanol extract and fractions

Extract/Fraction	Dose mg/kg body wt	Grams ± S.E.
Control	-	231.66 ± 16.13
Ethanol	300	478.0.28 ± 2.36
Pet. ether	300	594.00 ± 14.46
Solvent ether	300	451.33 ± 14.81
Ethyl acetate	300	504.33 ± 7.88
Butanol	300	601.66 ± 14.53
Butanone	300	532.00± 15.93

Conclusion

The recent studies on wound healing activity claim that flavonoids promote significant wound healing property[10-11]. The well-known property of almost every group of flavonoids is their capacity to act as antioxidants. Flavonoids protect the body against reactive oxygen species. Body cells and tissue are continuously threatened by the damage caused by free radicals and reactive oxygen species, which are produced during normal oxygen metabolism or induced by exogenous damage[12-14]. The increased production reactive oxygen species during injury result in consumption and depletion of the endogenous scavenging compounds and flavonoids may have an additive effect to the endogenous scavenging compounds. Flavonoids can also increase the function of endogenous antioxidants. It is also found that hydroxyl groups of flavonoids makes the radical inactive by combining with radical, Flavonoids [OH] + R → Flavonoids [O] + RH. From this study it is concluded that the wound healing activity of ***C. dichotoma*** fruits may be due to the presence of flavonoids.

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References

1. The Wealth of India, A Dictionary of Indian Raw Materials and Industrial Products—Raw Materials Series, Publication and Information Directorate, CSIR, New Delhi, 1950, Vol. 2, pp. 346-348.
2. Yoganarsimhan SN, Medicinal Plants of India, Interline Publishing Pvt. Ltd., Bangalore, India, 2000, Vol.1, pp. 138-140.
3. Rapisarda A, Ficarra R, Tommasin S, Caldbro ML and Hungsa S, *Cordia francisci, C. martinicensis, C. myxa, C. serratifolia* and *C. ulmifolia* Leaves as New Source of Rutine, Analgesic and Anti-inflammatory activity, *Planta Med*, 1992, **42**(2), 643-645.
4. Wassel G, El-Menshaw B, Saud A, Meharuna G and El-Merzabani M, Screening of Selected Plant for Pyrrolizidine Alkaloids and Antitumor Activity, *Pharmazie,* 1987, **42** (2), 709-711.
5. Rajesh MG, Paul B and Latha MS, Efficacy of Kamilari in Alcoholic Liver Cirrhosis, *Antiseptic,* 2000, **97** (2), 320-321.
6. Ghosh MN, Fundamentals of Experimental Pharmacology, Scientific Book Agency, Calcutta, 2nd edn, 1986, pp. 156-157.
7. Morton JJ, Evaluation of vulnerary activity by an open wound procedure in rats, *Arch Int Pharmacodyn,* 1996, **6** (1), 117-126.
8. Ehrlich HP and Hunt TK, Effects of Cortisone and Anabolic Steroids on the Tensile Strength of Healing Wounds, *J Ann Surg*, 1969, **170** (3), 203-206.
9. Lee KH, Study on the mechanism of action of Salicylates and retardation of wound healing by aspirin, *J Pharm Sci*, 1968, **57** (4), 1042-1044.
10. De Groot H, Reactive oxygen species in tissue injury, *Hepatogastroenterology*, 1994, **41**(2), 328-332.
11. Grace PA, Ischaemia-reperfusion injury, *Br J Surg,* 1994, **81**, 637-647.
12. Halliwell B, How to characterize an antioxidant: an update, *Biochem Soc Symp*, 1995, **61**, 73-101.
13. Korkina LG and Afanas'ev IB, Antioxidant and chelating properties of flavonoids, *Adv Pharmacol,* 1997, **38**, 151-163.
14. Hanasaki Y, Ogawa S and Fukui S, The correlation between active oxygens scavenging and antioxidative effects of flavonoids, *Free Radic Biol Med*, 1994, **16**, 845-850.
15. Kerry NL and Abbey M, Red wine and fractionated phenolic compounds prepared from red wine inhibit low density lipoprotein oxidation *in vitro*, *Atherosclerosis*, 1997, **135**, 93-102.