RESEARCH PAPER

Accepted 1 July 2003
Revised 2 May 2003
Received 21 June 2002
Indian J. Pharm. Sci., 2003, 65(4): 390-394

Wound Healing Potential of Some Active Principles of *Lawsonia Alba* Lam. Leaves

S. D. MANDAWGADE* AND KALPANA S. PATIL
Department of Pharmacognosy and Phytochemistry, K. L. E. S's College of Pharmacy,
J. N. M. C. Campus, Nehru Nagar, Belgaum-590 010.

Wound healing potential of different extracts of leaves of *Lawsonia alba* Lam. (Lythraceae) was evaluated on the rat excision and incision wound models. Different routes of administration (oral and topical) were studied in view to evaluate their efficacy in wound management. The naphthaquinone derivative, lawsone was isolated from the leaves and screened for same pharmacological activity. Results indicated that the oral administration of ethanol extract of *Lawsonia alba* leaves and lawsone exhibited significant healing response in both the wound models. Application of the same in the form of ointment was found to have better efficacy in wound repair compared to oral route. From the present study, it can be inferred that topical application of ethanol extract promoted the wound healing activity better than the other extracts.

Wound may be defined as a loss or breaking of cellular and anatomic or functional continuity of living tissues. In the traditional systems of medicine, various plants have been used to promote wound healing[1,2]. The leaves of *Lawsonia alba*, commonly called as Henna (*Mehendi*) are being used in the form of a decoction in the treatment of burns and skin inflammations. An ointment prepared from the leaves was used to cure wounds and ulcers[3,4]. The leaves have also shown to possess antifungal and antibacterial activities[5]. Henna is reported to contain a naphthaquinone, lawsone, which is a natural dye and mainly responsible for coloring the hair and skin of hands and feet. Lawsone has also been reported to be an immunostimulant[6]. One of the main constituents of the leaves of *Lawsonia alba* are flavonoids[7,8]. The present work has been taken up to study the possible effect of different extracts of the leaves of *Lawsonia alba* might have on wound healing process. The influence of these extracts on wound repair was also evaluated after administering these extracts through oral and topical routes.

MATERIALS AND METHODS

The leaves of *L. alba* were collected from local areas of Belgaum during November 2001 and were authenticated at Shri. B. M. K. Ayurved Mahavidyalaya, Shahapur, Belgaum.

Successive solvent extraction:

In the present study, around 500 g of air dried leaves of *L. alba* were reduced to a fine powder, which was subjected to hot continuous extraction in a Soxhlet extractor, successively with petroleum ether (40-60°), chloroform and ethanol (95%). Finally the powdered material was macerated with chloroform water for 24 h to obtain the aqueous extract. Each time before extracting with next solvent, the powdered material was dried in hot air oven below 50°. Each extract was then concentrated by distilling off the solvent followed by evaporation to dryness on a water bath. All extracts were kept in a desiccator and stored in a refrigerator for phytochemical and pharmacological studies. Each extract was subjected to qualitative chemical investigation for the identification of phytoconstituents such as sterols, glycosides, saponins, carbohydrates, alkaloids, flavonoids, tannins and proteins.

Method for isolation of lawsone[9]:

About 50 g of crushed fresh leaves of *L. alba* were extracted by agitation for 2 h with 200 ml of 20% sodium bicarbonate solution. The extract was filtered, marc was

*For correspondence
E-mail: sagar3003@rediffmail.com

reextracted with 100 ml of same solution for 1 h, filtered and the alkaline extracts were pooled together. The extract was acidified with dilute sulphuric acid and crude product obtained on standing was reextracted with sufficient quantity of ammonium hydroxide and again acidified with dilute hydrochloric acid. The product was finally extracted with two successive quantities of benzene (40 ml) and filtered. The filtrate was distilled to yield yellowish-brown coloured crystals of lawsone (mp-192-193°). Further it was characterized by UV and IR spectral analysis.

Method for preparation of ointments:

The simple ointment base[10] was chosen as a vehicle for the topical application of the extract and lawsone. One hundred milligrams of lawsone was mixed homogeneously with 100 g of simple ointment base USP to get lawsone ointment (0.1% w/w) and ethanol extract ointment (30% w/w) was prepared by incorporating 30 g of dried, powdered ethanol extract in 100 g of simple ointment base USP for the topical use.

Pharmacological screening:

Mice of either sex of 90 d age, weighing 20-25 g were used for acute toxicity studies. Wistar rats of either sex weighing 150-200 g were selected for the pharmacological study. The study protocols were approved by Animal Ethics Committee of the Institution (CPCSEA Registration No. 221).

Acute toxicity study:

The method of Miller and Tainter (1944)[11] was used for determination of lethal dose (LD_{50}). Gum acacia 1%, was used as a vehicle to suspend the various extracts and the suspension was administered orally. One tenth of the LD_{50} was used as the maximum dose to test the pharmacological effects possessed by the extract[12,13].

Wound healing studies:

Wistar rats were divided into nine groups consisting six in each and were labeled alphabetically from A to I. Animals were depilated at the desired site before wounding. They were housed individually with food and water given *ad libitum*, the basal food intake and body weights to the nearest gram were noted. The animals were starved for 12 h prior to study.

Excision wound:

It was inflicted on rats as described by Morton and Malone[14]. A circular wound of about 2.5 cm diameter was made on depilated ethanol-sterilized dorsal thoracic region of rats under light ether anesthesia and observed throughout the study. The observations of percent wound closure were made on day 4, 8 and 12 post wounding and also for epithelization period and size of scar area.

Resutured incision:

The method of Ehrlich and Hunt[15] was adopted. Under light ether anaesthesia, two paravertebral incisions of 6 cm were made through the entire thickness of the skin, on either side of the vertebral column of rats with the help of a sharp blade. The incisions were sutured using 4-0 size silk threads with the help of straight round bodied needle. On the eighth post wounding day, sutures were removed and the breaking strength was determined on 10th post wounding day by continuous constant water flow technique of Lee[16].

Drug treatment:

Group A, served as a control group and received single daily dose of vehicle (gum acacia 1%) orally. Groups B, C, D, E and F were treated groups and animals in these groups received a single daily oral dose of petroleum ether extract (154±7 mg/kg), chloroform extract (140±5 mg/kg), ethanol extract (154±7 mg/kg), aqueous extract (182±10 mg/kg) and lawsone (50±3 mg/kg), respectively. For topical application group G, served as a control group and 100 mg of vehicle (simple ointment base USP), was applied topically to them once a day. Group H and I were treated groups and received topical application of 100 mg in quantity of the ointment of ethanol extract and lawsone, respectively, once a day.

Statistical analysis:

All the results were analyzed by student's *t*-test and were expressed as mean±standard error of mean values. The level of significance was set at $p<0.05$.

RESULTS AND DISCUSSION

The average percent yield of petroleum ether, chloroform, ethanol and aqueous extracts were 3.93, 1.47, 31.0 and 5.31 w/w, respectively. Flavonoids (flavonoid glycosides), lawsone and tannins were found to be present in ethanol extract and steroids, saponins in various other extracts as observed by the qualitative chemical tests. The LD_{50} (mg/kg) values for various extracts are summarized in Table 1. To assess the wound healing activity of *L. alba* Lam. leaves, rat excision and incision wound models were selected and percent wound closure, time to epithelization, size of scar area and breaking strength of wound were studied. Two routes of drug administration (oral and topical) were

compared to evaluate efficacy in wound repair.

When compared to control (87.4±1.81%) on the d 12, lawsone, petroleum ether extract and aqueous extract of *L. alba* all showed significantly better ($p<0.05$) wound closure. A similar improvement in wound closure was seen with ethanol extract (97.3±0.55%) ($p<0.05$) compared to control. On the other hand, the chloroform extract (92.0±1.43%) failed to show any significant difference from the control (87.4±1.81%) (Table 2). The results indicate that ethanol extract and lawsone showed complete epithelization in 19±0.5 and 18.8±1.0 d, respectively (Table 2). It signifies better wound healing activity (decreased time of epithelization) ($p<0.05$). However, petroleum ether, chloroform and aqueous extracts have shown complete epithelization by d 19.3±0.7, 20.8±1.0, 20.6±0.7, respectively, which were also significantly ($p<0.05$) different from control group (23.5±0.4 d). The least scar areas were observed in the group treated with ethanol extract (10.3±1.21 mm^2), lawsone (10.4±1.14 mm^2), followed by petroleum ether extract, aqueous extract and chloroform extract. These were significantly decreased ($p<0.05$) when compared to control group with scar area of 31.2±1.57 mm^2. The results are given in Table 2.

TABLE 1: ACUTE TOXICITY STUDIES.

Extracts	LD_{50} (mg/kg)	1/10th of LD_{50} (mg/kg)
Petroleum ether (40-60°) extract	2200±101	220±10
Chloroform extract	1995±66	199.5±6.6
Ethanol extract	2200±101	220±10
Aqueous extract	2600±139	260±14
Lawsone	720±50	72.0±5.0

LD_{50} denote lethal $dose_{50}$.

The breaking strength in incision wound model was evaluated by continuous constant water flow technique. All five extracts showed increased mean breaking strength, compared to control (124±13.9 g), when given orally once a day. The maximum breaking strength was seen in groups treated with lawsone (215±12.8 g) and ethanol extract (214±12.9 g), which were statistically significant ($p<0.05$) from control. The petroleum ether extract and aqueous ex-

TABLE 2: EFFECT OF THE EXTRACTS OF *LAWSONIA ALBA* ON THE EXCISION WOUND PARAMETERS.

Group	Code given	% Wound contraction on			Period of epithelization (d)	Mean size of scar area (mm^2)
		Day 4	Day 8	Day 12		
ORAL TREATMENT						
Control	A	46.6±2.01	83.4±1.46	87.4±1.81	23.5±0.4	31.2±1.57
Petroleum ether (40-60°) extract	B	56.3±1.07*	91.2±1.58*	96.5±1.82*	19.3±0.7*	22.4±1.79*
Chloroform extract	C	58.3±2.54	85.4±2.80	92.0±1.43	20.8±1.0	26.3±1.62
Ethanol extract	D	60.9±2.39*	96.3±2.08*	97.3±0.55*	19.0±0.5*	10.3±1.21*
Aqueous extract	E	56.4±1.31*	88.4±1.99	94.4±1.26*	20.6±0.7*	24.0±1.81
Lawsone	F	61.5±2.32*	93.6±1.31*	96.0±0.64*	18.8±1.0*	10.4±1.14*
TOPICAL TREATMENT						
Control (simple ointment base USP)	G	43.8±1.24	74.3±1.05	77.6±1.17	22.6±0.5	39.0±2.03
Ethanol extract ointment (30%w/w)	H	40.8±4.86	95.9±0.59*	99.7±0.10*	16.0±0.4*	12.1±0.65*
Lawsone ointment (0.1%w/w)	I	39.9±1.09	95.0±0.51*	99.4±0.12*	17.2±0.4*	14.3±1.41*

All values are given in Mean±S.E.; *$p<0.05$ = Significant *Vs.* Control.

tract were also found to be significant ($p<0.05$). Mean breaking strength of chloroform extract (143±7.0 g) was little higher than control but was statistically insignificant ($p<0.5$). The results are summarized in Table 3.

The results of pharmacological screening implicated that ethanol extract and lawsone have greatly contributed towards wound healing activity ($p<0.05$), when given orally. Therefore, in view of ease of treatment and comparing the efficacy of oral and topical administration, both of these extracts were studied in excision and incision wound models by applying them topically in the form of ointment.

When compared to control (77.6±1.17%) on the d 12, ethanol extract (99.7±0.10%) and lawsone (99.4±0.12%) showed significant improvement ($p<0.05$) in percent wound closure when applied topically (Table 2). Significant decrease in time of epithelization ($p<0.05$) was observed in groups treated with ethanol extract of *L. alba* and lawsone ointment. The ethanol extract and lawsone showed complete epithelization in 16.0±0.4 and 17.2±0.4 d, respectively when compared to control (22.6±0.5 d, Table 2). This signifies better wound healing activity. The least scar areas observed for ethanol extract (12.1±0.65 mm^2) followed by lawsone (14.3±1.41 mm^2), which indicated a significant improvement ($p<0.05$) when compared to control (39.0±2.03 mm^2). The results are presented in Table 2.

TABLE 3: EFFECT OF THE EXTRACTS OF *LAWSONIA ALBA* ON THE BREAKING STRENGTH OF INCISION WOUNDS.

Group	Code given	Breaking strength (g)
ORAL TREATMENT		
Control	A	124±13.9
Petroleum ether (40-60°) extract	B	197±10.5*
Chloroform extract	C	143±7.0
Ethanol extract	D	214±12.9*
Aqueous extract	E	179±7.9*
Lawsone	F	215±12.8*
TOPICAL TREATMENT		
Control (simple ointment base USP)	G	125±6.2
Ethanol extract ointment (30% w/w)	H	444±7.5*
Lawsone ointment (0.1% w/w)	I	413±6.4*

All values are given in Mean±S.E.; *$p<0.05$ = Significant *Vs.* Control.

The maximum mean breaking strength was seen in the group treated with ethanol extract ointment (444.3±7.5 g) and followed by the group treated with lawsone ointment (413±6.4 g) (Table 3). These results strengthen the wound healing activity ($p<0.05$) for the ethanol extract of *L. alba*, when compared to control (125.2±6.2 g).

To find out most effective route for treatment, the results obtained by oral administration and topical application of ethanol extract and lawsone were compared with each other. The topical route of ethanol extract of *L. alba* and lawsone was found to significantly ($p<0.05$) promote the wound healing activity when compared to the ethanol extract and lawsone given by oral route (Tables 2 and 3). Even in comparison between topical application ethanol extract of *L. alba* and lawsone in the form of ointment, the ethanol extract was found effective ($p<0.05$) in promotion of wound healing than the lawsone ointment. The results are summarized in Table 4.

The phytochemical investigation of ethanol extract, revealed the presence of both flavonoids (flavonoid glycosides) and the lawsone. The flavonoid glycosides are well known for their role in wound healing[17]. Moreover, there are the reports that bioflavonoids have pharmacological activities such as antimicrobial[18] and antioxidant activities[19]. Lawsone is a polyphenolic compound[7]. The earlier reports on such polyphenols, revealed significant prohealing effect in both wound models used in this study[20].

The results of the present study have led to the conclusion that ethanol extract has exhibited more prominent wound healing activity than isolated lawsone and other extracts, when given by topical route. Similarly ethanol extract and isolated lawsone were also significantly better than other extracts when given by oral route. This improved wound promoting activity of ethanol extract could be attributed to the additional presence of lawsone along with the flavonoid glycosides, which as alluded earlier have significant wound healing activity. Further in our studies, it was found that the topical application of ethanol extract as well as isolated lawsone were more effective than the same given by the oral route.

TABLE 4: COMPARISON BETWEEN TOPICAL APPLICATION OF ETHANOL EXTRACT OINTMENT AND LAWSONE OINTMENT.

WOUND MODELS	EXCISION			INCISION
Parameters	% Wound closure on day 12	Period of epithelization (d)	Size of scar area (mm^2)	Breaking strength (g)
Control (simple ointment base USP)	77.6±1.17	22.6±0.5	39.0±2.03	125±6.2
Ethanol extract ointment (30% w/w)	99.7±0.10*	16.0±0.4*	12.1±0.65*	444±7.5*
Lawsone ointment (0.1% w/w)	99.4±0.12*	17.2±0.4*	14.3±1.41*	413±6.4*

All values are given in Mean±S.E.; *$p<0.05$ = Significant *Vs.* Control.

ACKNOWLEDGEMENTS

We thank the Principal, K. L. E. S's College of Pharmacy, Belgaum, for providing the facilities to carry out the research work. We also extend our thanks to Dr. S. K. Hiremath, for authentication of the plant.

REFERENCES

1. Udupa, S.L., Udupa, A.L. and Kulkarni, D.R., **Planta Medica,** 1991, 57, 325.
2. Bairy, K.L., **J. Nat. Remed.**, 2002, 2, 11.
3. Nadkarni, K.M., In; Indian Materia Medica, 3rd Edn., Vol. I, Popular Prakashan, Bombay, 1999, 730.
4. Dymock, W., Warden, C.J.H. and Hooper, D., In; Pharmacographica Indica, Vol. II, Periodical Experts, Delhi, 1976, 41.
5. Leung, A.Y. and Foster, S., In; Encyclopedia of Common Natural Ingredients Used In Food, Drugs And Cosmetics, 2nd Edn., A Wiley-Interscience Publication, New York, 1996, 297.
6. Kulkarni, S.R. and Karande, V.S., **Indian Drugs**, 1998, 35, 427.
7. Trease, G.E. and Evans, W.C., In; Pharmacognosy, 14th Edn., W.B. Saunders Company, London, 1997, 248.
8. Rastogi, R.P. and Mehrotra, B.N., In; Compendium of Indian Medicinal Plants, Vol. III, Publications and Information Directorate, New Delhi, 1993, 385.
9. Kokate, C.K., In; Practical Pharmacognosy, 4th Edn., Vallabh Prakashan, Delhi, 1994, 146.
10. Jamadar, M.G., Bairy, K.L. and Kulkarni, D.R., **Indian Drugs**, 1990, 27, 342.
11. Turner, R.A., In; Screening Methods In Pharmacology, 1st Edn., Academic Press, New York, 1965, 61.
12. Ghosh, M.N., In; Fundamentals of Experimental Pharmacology, Scientific Book Agency, Calcutta, 1971, 112.
13. Paget, G.E. and Barnes, J.M., In; Laurence, D.R., Eds., Pharmacometrics, Evaluation of Drug Activities, Vol. I, Academic Press, New York, 1983, 115.
14. Morton, J.J.P. and Malone, M.H., **Arch. Int. Pharmacodyn.**, 1972, 196, 117.
15. Ehrlich, H.P. and Hunt, T.K., **Ann. Surg.**, 1969, 170, 203.
16. Lee, K.H., **J. Pharm. Sci.**, 1968, 57, 1042.
17. Bairy, K.L. and Rao, C.M., **J. Nat. Remed.**, 2001, 1, 25.
18. Narayana, K.R., Reddy, S.M., Chaluvadi, M.R., Krishna, D.R., **Indian J. Pharmacol.**, 2001, 33, 2.
19. Robak, J. and Glyglewski, R.J., **Biochem. Pharmacol.**, 1988, 37, 837.
20. Padmaja, P.N., Bairy, K.L. and Kulkarni, D.R., **Fitoterapia**, 1994, 65, 298.