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Evaluation of anti-asthmatic activity of *Lawsonia inermis* Linn. Aerial parts

Snehal S Manekar and Manoj S Charde

Abstract

The aim of present study is to evaluate the antiasthmatic potential of aerial parts of plant of *Lawsonia inermis* Linn. Using several experimental models. The dried and powdered aerial parts of *Lawsonia inermis* was extracted with continuous soxhlet extraction with Petroleum ether (40-60^o C), Chloroform, Ethyl acetate, Acetone, Methanol and Hydroalcoholic solvents. Preliminary phytochemical screenings of all extracts were done. Preliminary animal studies by *In-vitro* isolated goat trachea chain preparation of all extracts were done to find out potent extract. In this study, the methanolic extract of aerial parts of *Lawsonia inermis* was found to be potent comparative to other extract. The results of carrageenan induced rat paw oedema model indicated the dose dependant anti-inflammatory activity. As compared to standard drug (Indomethacin), methanolic extract showed similar activity which were found to be statistically significant ($P < 0.0001$). The extent of DPPH radical scavenging was determined by calculated the IC₅₀ value of methanolic extract *Lawsonia inermis* (101.8) compared with the Ascorbic acid (114.7) taken as standard. In the present study the histamine induced dose dependent contraction of goat tracheal chain was significantly inhibited ($p < 0.001$) by methanolic extract of aerial parts of *Lawsonia inermis* (200 µg/ml). Thus the present study revealed that the methanolic extract of *Lawsonia inermis* (MEBP) has significant antihistaminic (H1 receptor antagonist) activity.

In view the fact that tribal have well experience the antiasthmatic effects of the roots of *Lawsonia inermis* Linn. The results of our study, for the first time, show that the methanolic extract of aerial parts of *Lawsonia inermis* Linn. Possesses antioxidant, anti-inflammatory, Bronchodilator properties and therefore can be used for the antiasthmatic treatment.

Keywords: Anti-asthmatic, Antihistaminic, Anti-inflammatory, *Lawsonia inermis*. Aerial parts

Introduction

Asthma is a dynamic, chronic disorder of the lungs involving airway obstruction, caused by inflammation and hyperresponsiveness. Asthma is a very common chronic condition. Its prevalence varies worldwide but more than 5% of any investigated population suffers from asthma. In some regions this percentage is much higher. Asthma affects all ages: it is the most common chronic disease of childhood, adolescence and adulthood and affects patients in their most productive years^[1].

The use of *Lawsonia inermis* L. (henna) for medicinal and cosmetic purposes is inextricably linked to ancient and modern cultures of Asia. It has been traditionally reported in use of headache, hemicranias, lumbago, bronchitis, boils, ophthalmia, syphilis, sores, amenorrhea, scabies, diseases of the spleen, dysuria, bleeding disorder, skin diseases, diuretic, antibacterial, antifungal, anti-amoebiasis, astringent, anti-hemorrhagic, hypotensive and sedative effect. The plant is reported to contain Lawsone, Esculetin, Fraxetin, Isoplumbagin, Scopoletin, Betulin, Betulinic acid, Hennadiol, Lupeol, Lacoumarin, Laxanthone, Flavone glycosides, two pentacytic triterpenes^[2].

Materials and methods

Plant materials

The aerial parts of plant *Lawsonia inermis* were collected from the outfield of Amravati city, Maharashtra, India in month of November- December. The plant materials (Ref. No-Gvish/BOT/Report/10/2015) were identified and authenticated by Dr. S.N. Malode, Head, P.G. Department of Botany, Govt. Vidarbha Institute of Science and Humanities, Amravati (M.S.), India.

Preparation of extract of *Lawsonia inermis* linn.

The dried and powdered aerial parts of *Lawsonia inermis* (500 gm) were extracted with

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Continuous soxhlet extraction with Petroleum ether (40-60°C), Chloroform, Ethyl acetate, off the solvents and evaporated to dryness using water bath to get crude extract. All extracts were dried.

Experimental animals

For anti-inflammatory activity, Wistar albino rats weighing 140–160 g of either sex were used. Six animals were group housed in polypropylene cages (640 x 410 x 250 mm high) and kept in well cross ventilated room at the same experimental condition explained above. They were provided with standard rodent pellet diet and tap water *ad libitum* except the food was withdrawn 18–24 h before the experiment [4]. All the experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC), Government College of Pharmacy, Amravati, India (approved number-1370/ac/10/CPCSEA).

Acute toxicity test (Determination of LD50)

The crude methanolic extracts and isolated compound were used for the test. Wistar rats (200–250 g) of either sex were used. This method involved an initial dose finding procedure, in which the animals were divided into five groups of three animals per group. Doses of 200, 600, 1000, 1500 and 2000 mg/kg were administered orally, one dose for each group. The treated animals were monitored for 24 h for mortality and general behavior [5].

Phytochemical screening of extracts of *Lawsonia inermis* linn.

All extracts of *Lawsonia inermis* were subjected to phytochemical screening for tannins, glycosides, steroids, terpenoids, flavonoids and alkaloids according to the methods of Trease and Evans.

Screening for antiasthmatic activity

Preliminary animal study by *In-vitro* isolated goat trachea chain preparation of all extracts of *Lawsonia inermis* Linn.

Isolated adult goat tracheal tissue was obtained immediately after slaughter of the animals. Trachea was cut into individual rings and tied together in series to form a chain. Trachea was suspended in bath of Kreb's solution and was continuously aerated at $37 \pm 0.5^\circ\text{C}$. Dose–response curve (DRC) of histamine in plain Kreb's solution and in different doses of *Lawsonia inermis* different extracts viz., Pet. Ether, Chloroform, Ethyl acetate, Acetone, Methanol and Hydroalcoholic in Kreb's solution were taken. Graph of percentage of maximum contractile response on ordinate and concentration of histamine on abscissa was plotted to record DRC of histamine, in absence and in the presence of drug extracts.

Isolation of phytoconstituent from selected extract

Column chromatographic separation was done to isolate phytoconstituents from methanolic extract of *Lawsonia inermis* which had shown significant Antihistaminic activity than other extract. The concentrated methanolic extract (20 g) was chromatographed through a column of silica gel 60-120

mesh L. R. (diam. 4cms X length 45 cms). The column being successively eluted with increasing polarities of chloroform (100%), chloroform: Ethyl acetate (95:5 to 5:95), Ethyl acetate (100%), Ethyl acetate: Methanol (90:10 to 10:90) and methanol (100%). A total number of 157 fractions were obtained. Those consecutive fractions, which have the same number of spots with the same Rf values, were combined and evaporated to dryness to get four major fractions.

Table 1: Isolated fractions from methanolic extract of *Lawsonia inermis*.

Fraction	No. of collections	No. of spots
F1	22-36	1
F2	37-45	2
F3	46-63	3
F4	64-76	3

Antioxidant activity

In Vitro DPPH radical scavenging activity

The free radical scavenging activity of various extract can be measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH. Prepare 0.1mM solution of DPPH in Methanol and add 1.0 ml of this solution to 3.0 ml of methanolic *Lawsonia inermis* extract and isolated fraction (F1LI and F2LI) in different concentrations (25-800 µg/ml). Thirty minutes later, measure the absorbance at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity.

Preparation of extract dilution

50 mg of *Lawsonia inermis* methanolic extract was weighed separately and dissolved in 100 ml of methanol to get 500 µg/ml stock solutions. Lower concentrations (25, 50, 100, 150, 200, 250 µg/ml) were prepared by diluting serially with methanol.

Preparation of standard dilution

Ascorbic acid weighed (50 mg) separately and dissolved in 100 ml of methanol to get 500 µg/ml stock solutions. Lower concentrations (50, 100, 150, 200, 250 µg/ml) were prepared by diluting serially with methanol.

Anti-inflammatory activity

Carageenan induced paw edema in rat

Carrageenan induced hind paw edema model can be used for determination of anti-inflammatory activity. 60 min after the oral administration of methanolic *Lawsonia inermis* extracts (MELI 200 mg and 400 mg/kg) and fractions LI (F1LI and F2LI 200 and 400µg/kg) allow each rat to inject with freshly prepared suspension of carrageenan (0.5 mg/25 µl) in physiological saline into sub plantar tissue of the right hind paw. As the control, Inject 25 micro liter saline solutions into that of the left hind paw. Measure the Paw edema in the intervals of 0, 1, 2,3,4,5 and 6 hr. after induction of inflammation. Measure the difference in footpad thickness. Compare the mean values of treated groups with those of a control group and analyzed by using statistical methods. Use Indomethacin (10 mg/kg) as the reference drug.

Table 2: Experimental Design: Carageenan induced paw edema in rat.

Group	Treatment	Dose
I	Control (Normal saline)	2 ml/Kg b. wt
II	Standard Indomethacin	10 mg/Kg b. wt
III	Methanolic extract of <i>Lawsonia inermis</i> (Lower)	200 mg/Kg b. wt

IV	Methanolic extract of <i>Lawsonia inermis</i> (Higher)	400 mg/Kg b. wt
V	Fraction 1 <i>Lawsonia inermis</i> (Lower) (F1LI)	200 µg/ml
VI	Fraction 1 <i>Lawsonia inermis</i> (Higher)	400 µg/ml
VII	Fraction 2 <i>Lawsonia inermis</i> (Lower) (F2LI)	200 µg/ml
VIII	Fraction 2 <i>Lawsonia inermis</i> (Higher)	400 µg/ml

Bronchodilator activity

Inhibition of histamine induced contraction in isolated goat tracheal chain preparation. (*In-vitro* model)

Isolated adult goat tracheal tissue was obtained immediately after slaughter of the animals. Trachea was cut into individual rings and tied together in series to form a chain. Trachea was suspended in bath of Kreb's solution and was continuously aerated at 37 ± 0.5°C. Dose-response curve (DRC) of histamine in plain Kreb's solution and in different doses of methanolic extract of *Lawsonia inermis* and isolated fraction (F1LI), (F2LI) in Kreb's solution were taken. Graph of percentage of maximum contractile response on ordinate and concentration of histamine on abscissa was plotted to record DRC of histamine, in absence and in the presence of drug extracts.

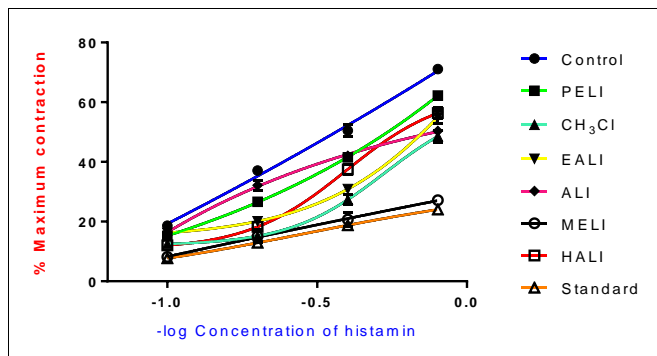
Statistical analysis

The statistical analysis was performed by using one way analysis of variance (ANOVA) followed by Dunnett's test for individual comparison of groups with the control. The p values less than 0.001 were considered as significance.

3. Results and discussion

Preliminary animal study by *In-vitro* isolated goat trachea chain preparation of all extracts of *Lawsonia inermis*.

It was observed that methanolic extract of *Lawsonia inermis* (MELI) and hydroalcoholic extract (HALI) inhibits contraction produced by histamine in these tissue preparations as compared to the pet ether, chloroform, ethyl acetate, acetone extracts of LI. Histamine (30µg/ml) was taken in different dose level and DRC was plotted. Study revealed that Methanolic and hydroalcoholic extracts both exhibits significant ($p < 0.001$) percentage decreased contraction at concentration 200 µg /ml in goat tracheal chain preparation. From phytochemical screening methanolic extract was selected for further study.



Graph 1: Effect of all extracts of aerial parts of *Lawsonia inermis* on histamine induced bronchospasm

Values are expressed as mean ± SEM *** $p < 0.001$; ns = non significant, compared with standard Group (one-way ANOVA followed by Dunnett's Multiple Comparisons test).

Acute toxicity study of aerial parts methanolic extract of *Lawsonia inermis* linn.

The methanolic extract of *Lawsonia inermis* (MELI) and isolated fraction F1LI and F2LI were administered to group of experimental animals at doses that is 200, 400...and 2000 mg/kg and 200.1000 µg/kg respectively were selected for this study. The acute oral toxicity study was carried out as per the OECD guidelines found that both methanolic extract and isolated fraction were safe at limit dose of 2000 mg/kg with no mortality in studied animals. 1/10th of these doses i.e. 200 mg/kg and doubling of that dose that is 400 mg/kg and half of 1/10 dose that is 100 mg/kg were used in the subsequent study respectively.

Phytochemical screening of extracts of *Lawsonia inermis* linn.

In phytochemical screening methanolic LI extract contain Alkaloids, Saponin, Glycoside, Flavonoid, Phytosterol, Protein, Carbohydrate.

Isolated Compound from Aerial Parts of *Lawsonia inermis* Linn.

TLC profiling of isolated compound (F1LI and F2LI) from aerial parts of *Lawsonia inermis* linn.

Fraction F3 and F4 were eluted from Ethyl acetate: Methanol (60:40) and Ethyl acetate: Methanol (50:50) respectively, resulted as a single compound, which was confirmed by TLC (CHCl₃: MeOH; 7:3). The product was designated as F1LI and F2LI. Visualization was carried out by spraying 10% methanolic Sulphuric acid reagent and detection was carried out visually in visible light and under UV light. Dipping the plate in iodine chamber also shows color spot. Rf value of F1LI was found to be 0.62 and F2LI have 0.95.

Antioxidant Activity

***In Vitro* DPPH radical scavenging activity**

The result of DPPH scavenging activity assay in this study indicates the methanolic extract was potentially active. The scavenging activity of methanolic extract compared with the standard drug ascorbic acid suggested that the plant is also a potent scavenger of free radicals. The antioxidant activity on the basis of their IC₅₀ values was methanolic LI extract (IC₅₀ 101.8 µg/ml) and isolated compound F1LI (96.18 µg/ml) and F2LI (97.13 µg/ml). Results were compared with ascorbic acid (IC₅₀ 114.7µg/ml).

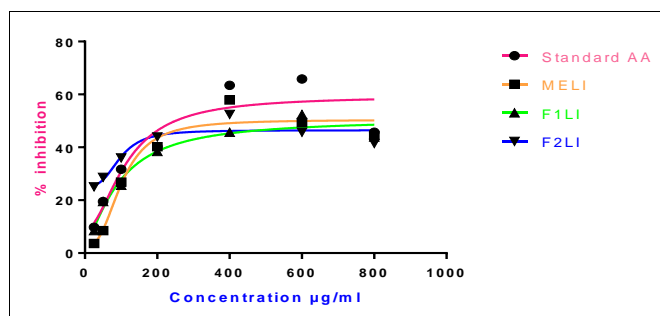
Table 3: Percent inhibition of DPPH absorbance at different concentrations of standard (ascorbic acid)

Concentration (µg/ml)	% Inhibition			
	MELI	F1LI	F2LI	Standard
25	3.65	8.53	25	9.75
50	8.53	19.51	28.65	19.51
100	26.82	25.60	35.97	31.70
200	40.24	38.41	43.90	40.24

400	57.92	45.73	52.43	63.41
600	49.39	52.43	45.73	65.85
800	44.51	80.48	41.46	45.73

Absorbance of control: 0.164

Values are expressed as mean ± SEM, ** $p < 0.01$, *** $p < 0.001$; ns =non-significant, compared with Standard (one-way ANOVA followed by Dunnett’s Multiple Comparisons test).



Graph 2: Effect of *Lawsonia inermis* Methanolic extract and isolated fraction on DPPH Antioxidant assay

Values are expressed as mean ± SEM, ** $p < 0.01$, *** $p < 0.001$; ns =non-significant, compared with Standard (one-way ANOVA followed by Dunnett’s Multiple Comparisons test).

Table 4: Comparison of IC₅₀ of extracts and isolated fraction from *Lawsonia inermis*

<i>L. inermis</i> Extract	IC ₅₀ µg/ml
Standard Ascorbic acid	114.7
MELI	101.8
F1LI	96.18
F2LI	97.13

Mean values ($P < 0.001$) according to Duncan's Multiple Range Test.

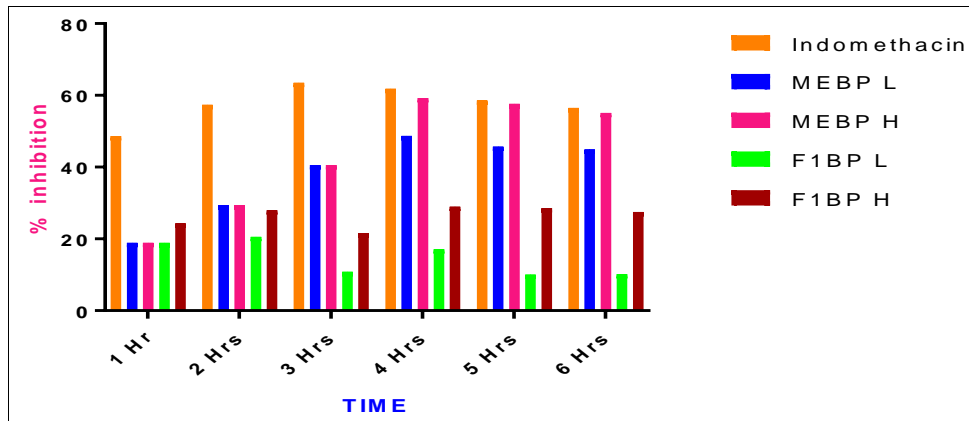
Anti-inflammatory activity
Caragenan induced paw edema

Table 5: Anti-inflammatory activity of methanolic extract of *Lawsonia inermis* L. by carrageen induced rat paw edema

Group No.	Group	Dose mg/Kg	% Inhibition					
			1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
I	Indomethacin	10	48.64	57.35	63.51	61.84	58.57	56.52
II	MELI Lower	13.04	10.81	17.64	10.81	28.94	25.71	27.53
III	MELI Higher	47.82	29.72	32.35	14.86	32.89	31.42	40.57
IV	F1LI Lower	8.69	8.10	23.52	6.75	11.84	17.14	24.63
V	F2li Higher	26.08	45.94	39.70	18.91	18.42	28.57	26.08
VI	F2LI Lower	8.69	27.02	7.35	16.21	30.26	28.57	30.43
VII	F2LI Higher	26.08	54.05	20.58	5.40	11.84	12.85	15.94

In the caragennan induced rat paw edema test (table-I, fig-I &II), methanolic extract of LI (MELI 200 mg/kg) & isolated compound (F1LI, F2LI 200 µg/kg) showed significant (** $P < 0.001$), with 32.89 % & 11.84, 30.26 % inhibition of edema respectively at the end of 4 h. At a dose of 400 mg/kg methanolic extract of LI (MELI) & isolated compound (F1LI, F2LI) showed significant (** $P < 0.0001$) with 32.89% & 18.42, 11.84% inhibition of edema respectively at the end of 4 h as compared with reference drug indomethacin (** $P < 0.0001$) with 61.84 % inhibition of edema. The caragennan induced rat paw edema is a biphasic process [19, 20]. From the result methanolic extract of LI (MELI) lower and

higher dose showed approximately similar activity (28.94 and 32.89 % respectively). It means by increasing in dose there is no effect on action. While in isolated fraction F1LI do not shown satisfactory % inhibition of paw edema (F1LI H 18.42%). F2LI lower dose (200 µg/kg) showed maximum % inhibition of paw edema (30.26%) than higher dose (400 µg/kg) In all MELI higher dose and isolated fraction F2LI lower dose showed similar % inhibition against caragennan induced paw edema which is comparable with reference standard Indomethacin.



Graph 3: Effect of *L. inermis* Methanolic extract and isolated fraction on Carrageenan Induced Rat Paw Oedema

Values are expressed as mean ± SEM (n = 6), ** $p < 0.01$, *** $p < 0.0001$; ns = non significant, compared with Disease Control Group (one-way ANOVA followed by Dunnett’s Multiple Comparisons test).

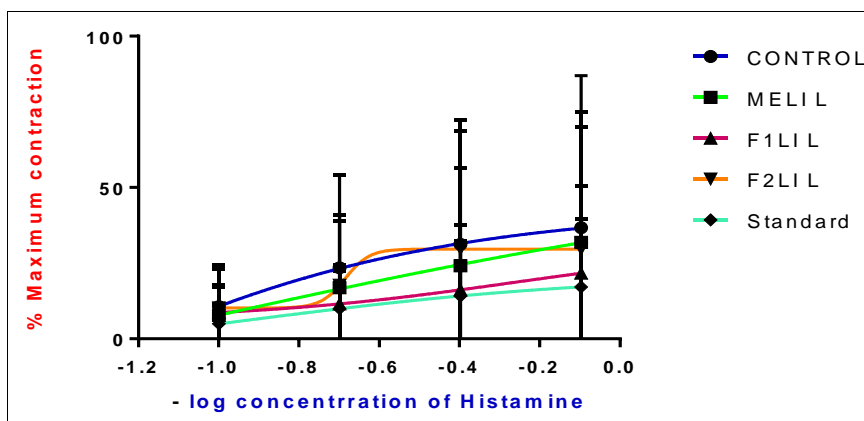
Bronchodilator activity
Inhibition of histamine induced contraction in isolated goat tracheal chain preparation. (In-vitro model)

It was observed that methanolic extract of *Lawsonia inermis* Linn. inhibits dose dependent contraction produced by histamine (30 µg/ml) as indicated in the graph of maximum percentage of contractile response v/s negative log molar concentration of histamine. Study revealed that methanolic extract of *Lawsonia inermis* exhibits significant ($p < 0.001$) percentage decreased contraction at concentration 200 µg/ml in goat tracheal chain preparation

Table 6: Effect of *Lawsonia inermis* methanolic extract on histamine induced contraction on isolated goat tracheal chain preparation.

Dose of Histamine (30 µg/ml)	% maximum contraction (Mean ± SEM)				
	Control group	MELI	F1LI	F2LI	Standard
0.1	20.42±0.89	14.32±1.02	15.08±2.30	19.36±0.98	9.62±0.26
0.2	45.14±1.75	32.63±1.12	20.69±2.38	34.27±1.13	18.73±1.14
0.4	60.4±2.02	47.13±1.19	31.27±1.10	57.35±2.13	27.16±1.16
0.8	72.27±1.14	62.48±1.32	42.17±1.18	58.09±1.09	33.14±1.16

Each value represents the mean ± S.E.M.; ns Not significant, * $P < 0.05$, significant; *** $P < 0.0001$ vs. control (histamine), one way ANOVA followed by Dunnett’s *t*-test.



Graph 4: Bronchodilator Activity of *L. inermis* extract and isolated fraction (Inhibition of tracheal tissue contraction).

Values are expressed as mean± SEM ** $P > 0.001$ significant compared to control group by one way ANOVA followed by Dunnett’s multiple comparison test.

Conclusion

The results of the investigation revealed that methanolic extracts of the *L. inermis* showed significant DPPH radical activity which was calculated in terms of IC50. There was no major difference in the DPPH radical scavenging activity of methanolic extract (MELI and isolated compound F1LI). From the present study, it is concluded that methanolic extract of *L. inermis* showed maximum percentage inhibition (32.89%) of rat paw edema at a dose of 400 mg/kg. Anti-

inflammatory activity was found to be dose dependant. In case of isolated fraction F2LI lower dose showed significant activity than F1LI.

Histamine contracts the tracheo-bronchial muscle of guinea pig, goat, horse, dog and man. Goat tracheal chain is easier to handle and to prepare; it is also much more sensitive than guinea pig tracheal chain. The goat tracheal muscle has H1, M3 and B2 receptors. The stimulation of H1 receptors caused contraction of bronchial smooth muscle. In the present study, there is right side shift of Dose Response Curve (DRC) of histamine in the presence of methanolic extract of *Lawsonia inermis* indicating antiasthmatic action.

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