

Synergistic anti-inflammatory Activity of *Lawsonia Inermis* linn. and *Chlorophytum Brovilianum* sant

*Nitu Singh¹, Praveen Kumari²

1. Department of Pharmacognosy, NIMS Institute of Pharmacy, NIMS University, Jaipur, Rajasthan-303121, India.
2. S.S.D Institute of Pharmacy and Medical Technology, Jhajjar, Haryana-124103, India.

ABSTRACT

Nonsteroidal and steroidal drugs are generally used to treat inflammation. However, these drugs have side-effects like nausea, vomiting, etc. This lead to search for new anti-inflammatory agents from natural sources, which would be effective and safe. Many plant extracts show a synergistic effect with each other or with modern drugs. The aim of the present study is to evaluate the synergistic anti-inflammatory activity of chloroform extracts of leaves and roots tubers of *Lawsonia inermis* Linn. and *Chlorophytum borivilianum* Sant. respectively in albino wistar rats. The synergistic anti-inflammatory activity is evaluated by using plethysmometer. The study is carried out using dose of 200 mg/kg orally for both the extract individually and for combined extract. Both the chloroform extracts produced statistically significant and dose dependent inhibition of edema induced by Brewer's yeast-induced rat paw edema and Cotton pellet induced granuloma at all doses when compared to the control groups. The percentage inhibition is also noted for the combination of chloroform extract. Both the extracts in combination did not exhibit toxicity in mice when given orally up to the concentration of 2000mg/kg. Statistically processed results support the conclusion, that the combination of extract of *Lawsonia inermis* Linn. and *Chlorophytum borivilianum* Sant. (1:1) possesses dose dependent, significant synergistic anti-inflammatory activity against experimentally induced inflammation.

Keywords: Anti-inflammatory, *Chlorophytum borivilianum* Sant., indomethacin, *Lawsonia inermis* Linn., plethysmometer

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*Address for correspondence:

Nitu Singh,

Assistant Professor,

Department of Pharmacognosy, NIMS Institute of Pharmacy, NIMS University, Jaipur, Rajasthan, India.

E-mail: nanhushagun@gmail.com

INTRODUCTION

The NSAIDs are one of the most widely prescribed drugs in the world and are extensively used to alleviate clinical cases specially for pain and inflammation [1]. However, these drugs are well-known to induce stomach ulceration and delay ulcer healing [2]. Despite recent advances, an adequate remedy for the NSAID-induced gastropathy remains elusive. The World Health Organization has stressed the need to develop drugs from plant origin, which will be inexpensive, accessible particularly to the rural people in the developing countries, and show less/no side effects. Therefore, development of a drug from plant sources without compromising the efficacy and safety would be expected to

benefit millions of suffering humanity. Many plant extracts show a synergistic effect with each other or with modern drugs. *Lawsonia inermis* Linn. commonly known as *Henna* belongs to family Lythraceae (**Fig. 1**). It is a glabrous branched shrub or small tree (2 to 6 m in height) [3]. The reported chemical constituents of this plants are Lawsone (2-hydroxy 1,4-naphthoquinone), luteolin-7-glucoside, luteolin-3-glucoside, linalool, α ionone, β ionone, α -terpineol, terpinolene, δ -3-carene and γ -terpineol, Gallic acid [4,5]. The various ehtanomedicinal use of *Lawsonia inermis* are diuretic, emetic, edema, expectorant, constipating, liver tonic, haematinic, wound, ulcers, cough, bronchitis, burning sensation,

inflammations, diarrhoea, fever, falling of hair, greyness of hair, jaundice [6,7]. *Chlorophytum borivillianum* Sant. commonly known as Safed moosli belongs to the family Liliaceae (**Fig. 2**). It grows to 10–60 cm tall, with a rosette of long, slender leaves 15–75 cm long and 0.5–2 cm broad, growing from a thick, fleshy rhizome [8]. It is a rich source of alkaloids, vitamins, minerals, proteins, carbohydrates, steroid and polysaccharides. Saponin, sapogenin (hecogenin), fructans, simple sugars such as glucose, fructose, phenolics, triterpenoids, gallo-tannins, mucilage, zinc, copper and phosphorus [9,10]. The ethnomedicinal uses are Aphrodisiac, Sexual stimulant, Natural sex tonic, Adaptogen, Metabolic regulator, Anabolic effects, Libido, arousal, spermatogenesis, Antioxidant, Anti-stress, and Antibacterial effects, Anti-ulcer, Analgesic, Anti-obesity, and lipid-regulation properties, anti-inflammatory [11]. As the ethnomedicinal use both the plants showing anti-inflammatory effect. The extracts of *Lawsonia inermis* Linn. and *Chlorophytum borivillianum* Sant. have been reported to possess anti-inflammatory activity [12–15]. We conducted this study to determine the synergistic anti-inflammatory activity of both crude drug extract when given in combination.



Figure 1: Plant of *Lawsonia inermis* Linn



Figure 2: Root tubers of *Chlorophytum borivi.* Sant [16]

MATERIALS AND METHODS

Plant material

The roots tubers of *Chlorophytum borivillianum* Sant. and leaves of *Lawsonia inermis* Linn. were collected from the local market of Haryana and were authenticated by NISCAIR.

(NISCAIR/RHMD/CONSULT/2011-12/1985/ 285). The leaves and roots tubers were washed, dried and powdered. Both the powdered drug is defatted by petroleum ether. Thereafter both the dried plant parts were extracted with chloroform for 72 hrs by maceration & soxhlation respectively.

Animals

Albino Wister rats (male) 180-200 gm were used. The animals were kept in the standard polypropylene cages and provided with food and water *ad libitum*. The animals were acclimatized for a period of 14 days prior to performing the experiments. The experimental protocols were approved by Institutional Animal Ethics Committee (NU/PH/M/COG /12/78).

Preparation of anti-inflammatory agent

Brewer's yeast (0.1 ml of 20% suspension in 0.9% saline)

Preparation of standard drugs:-

Indomethacine at dose of 10 mg / kg body weight was used as standard drug [17].

Preparation of test drug

Lawsonia inermis and *Chlorophytum borivillianum* extracts were dissolved in 1% & 2% gum acacia in distilled water respectively [18–20].

Lethal Dose (LD 50) [21–22]

Rats administered with doses ranges from (175 to 2000 Mg/Kg) were observed for any clinical symptoms changes in physical activities, mortality rates. Any sign of change in mental or physical activities were recorded and the number of death in each group (if any) within 24 h was recorded for 10 days. The groups were not shown any mortality within 7 days were further observed for 3 more days for any sign of delayed toxicity .Up to 2000 mg/kg *C.borivillianum*, *Lawsonia inermis* and combination of these two drugs were not shown any mortiliay rate.

Anti-Inflammatory Activity [23–24]

Brewer's yeast-induced rat paw edema

The anti-inflammatory activities were evaluated by using brewer's yeast induced

rat paw edema method. The animals were divided into five groups of six animals each. All drugs were given orally to the respective groups one hour before brewer's yeast (0.1 ml of 20% suspension in 0.9% saline) injection. I group serving as control received brewer's yeast (0.1 ml of 20% suspension in 0.9% saline). II group served as positive control and received Indomethacin 10 mg/kg body weight. III and IV groups were receiving the chloroform extracts of *Lawsonia inermis* Linn and *Chlorophytum borivilianum* Sant. (200 mg/kg). The V group received combination of above two drugs (1:1). Food was withdrawn over night, but adequate supply of water was given to the rats before the experiment. The drugs were given orally with the help of an oral catheter. The paw volume were measured for 0,1,2,3,4,5,6,24 hours. The average paw swelling in a group of extracts treated rats were compared with control group (treated with vehicle) and the standard (indomethacin). Percentage inhibition was calculated by using the formula.

$$\% \text{ inhibition} = \frac{V_c - V_t}{V_c} \times 100$$

Where, V_c = edema volume of Control,

V_t = edema volume of the test.

Cotton pellet induced granuloma [25-28]

Wistar albino rats male 170 -200 gm were divided into 5 groups of 6 animals in each group. The groups were fasted overnight. Cotton pellets weighing 30 ± 1 mg were sterilized in hot air oven and implanted subcutaneously into both sides of the axillae and groin region of each rat. Group I served as control and received the vehicle. Group II animals received indomethacin at a dose of 10 mg/kg orally for same period. The Extracts of *Lawsonia inermis* Linn and *Chlorophytum borivilianum* Sant. at concentration 200mg/kg were administered orally for Group III and IV animals for 9 days. Group V were received combination of above two extract (1:1). On the 10th day the animals were sacrificed and the pellets together with the granuloma tissues were carefully removed, dried in an oven at 60°C for 24 hrs, weighed and compared with control. Increment in the dry weight of the pellets is taken as a measure for granuloma formation.

Indomethacin (10 mg / kg / p.o.) was used as reference standard.

Statistical analysis

All data are expressed as mean \pm S.E.M, and statistical significance was determined via Dunnett's test by using graph pad prism 5.

RESULTS

Brewer's yeast-induced rat paw edema

The results of orally administered drugs influencing Brewer's yeast-induced paw oedema are summarized in (Table 1). The chloroform extract of both the leaves (*L. inermis*) and root tubers (*C. borivilianum*) showed significant reduction in the edema volume at a dose of 200 mg/kg body weight, which is comparable to standard drug indomethacin. Combination of both the extract showed maximum anti-inflammatory activity. Both extract showed significant anti-inflammatory activity at 24 hr. The percentage of paw edema was found to be better with the *Lawsonia inermis* Linn. extract than the *C. borivilianum* Sant. extract. The activity may be attributed due to the presence of flavanoids and Tannins [29].

Cotton pellet induced granuloma

The results of the chloroform extract on granuloma formation in the cotton pellet method are presented in (Table 2). Chloroform extract of both the drug at the test dose of 200mg/kg body weight caused significant reduction in the weight of the cotton pellet granuloma in rats as shown in (Table 2). The standard drug indomethacin caused 57.63% & 50.45% inhibition as compared to the control.

DISCUSSION

Brewer's yeast-induced rat paw edema

The development of edema in the paw of the rat after the brewer's yeast is due to release of histamine, serotonin and prostaglandin like substances [30]. Our results revealed that administration of chloroform extract inhibited the edema starting from the first hour and during all phases of inflammation, which is probably due to inhibition of different aspects and chemical mediators of inflammation.

Cotton pellet induced granuloma

Inflammation involves the proliferation of the macrophages, neutrophils and fibroblast, which are the basic source of granuloma formation. Therefore decrease in granuloma weight indicates the

suppression of the proliferative phase [31]. Which was effectively inhibited by chloroform extract of both the drugs. The present study has been shown that the

combination of *Lawsonia inermis* Linn. & *Chlorophytum borivilianum* Sant. possess more anti-inflammatory activity.

Table 1: Brewer's yeast induced paw edema model

Group (n=6)	Treatment & dose	Paw volume (% inhibition)						
		1 hr	2hr	3hr	4hr	5hr	6hr	24 hr
Control		0.63±0.05 —	0.95±0.07 —	1.3±0.07 —	1.58±0.07 —	1.81±0.09 —	2.11±0.12 —	1.83±0.18 —
Standard drug	10 mg/kg	0.48±0.03* (23.69%)	0.6±0.07*** (36.84%)	0.75±0.04*** (43.30%)	0.9±0.06*** (43.14%)	1.31±0.20% (25±0.05)***	1.52±0.04*** (28.34%)	1.15±0.09*** (37.26%)
Lawsonia inermis Linn	200mg/kg	0.57±0.06 (10.42%)	0.63±0.09*** (28.10%)	0.78±0.05*** (39.76%)	0.93±0.06*** (41.06%)	1.27±0.07*** (30.26%)	1.55±0.06*** (26.78%)	1.18±0.76*** (35.46%)
Chlorophytum borivilianum Sant	200mg/kg	0.53±0.02 (15.79%)	0.68±0.09*** (28.10%)	0.8±0.04*** (38.46%)	0.97±0.06*** (38.91%)	1.3±0.06*** (28.45%)	1.50±0.06*** (27.58%)	1.21±0.07** (33.60%)
Combination	200mg/kg	0.53±0.02 (15.79%)	0.62±0.03*** (35.05%)	0.78±0.05*** (39.76%)	0.93±0.06*** (41.06%)	1.28±0.06*** (29.39%)	1.57±0.06*** (25.98%)	1.17±0.06*** (35.46%)

Values are mean±S.E.M. (n = 6), Experimental groups were compared with control *(P<0.05), ***(P<0.001)

Table 2: Cotton pellet induced granuloma

S.no	Treatment	Wt of dry granuloma	%Inhibition of dry granuloma	Wt.of wet granuloma	% inhibition of wet granuloma
1	Control	49.17±2.386	—	186.7± 6.146	—
2	Indomethacin	20.83±1.537***	57.63	92.50± 3.819	50.45***
3	L.inermis Linn	22.17±1.138***	54.91	105.0 ±3.162	43.76***
4	C.borivilianum	22.67±2.060***	53.89	102.5 ± 2.141	45.09***
5	Combination	21.83±2.522***	55.6	98.33±5.270	47.33***

Values are mean±S.E.M. (n = 6) Experimental groups were compared with control *(P<0.05), ***(P<0.001)

CONCLUSION

Both the chloroform extracts produced statistically significant and dose dependent inhibition of edema induced by Brewer's yeast-induced rat paw edema and Cotton pellet induced granuloma at all doses when compared to the control groups. Statistically processed results support the conclusion, that the combination of extract of *Lawsonia inermis* Linn. and *Chlorophytum borivilianum* Sant. (1:1) possesses dose dependent, significant synergistic anti inflammatory activity against experimentally induced inflammation. The synergistic activity of both the extract is due to multiconstituent synergism.

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