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

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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 synergestic anti-inflammatory activity against experimentally induced inflammation.

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

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. commonaly 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 borivilianum* 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 ethanomedicinal 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 ethanomedicinal use both the plants showing anti-inflammatory effect. The extracts of *Lawsonia inermis* Linn. and *Chlorophytum borivilianum* 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.

**MATERIALS AND METHODS**

**Plant material**
The roots tubers of *Chlorophytum borivilianum* 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 & soxhletation 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 borivilianum* 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.borivilianum, *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 Chlorophytm 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, Vc= edema volume of Control, Vt = 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±1mg 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 Chlorophytm 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±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 & Chlorophyrum borivilianum Sant. possess more anti-inflammatory activity.

Table 1: Brewer’s yeast induced paw edema model

<table>
<thead>
<tr>
<th>Group (n=6)</th>
<th>Treatment &amp; dose</th>
<th>Paw volume (% inhibition)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 hr</td>
<td>2 hr</td>
</tr>
<tr>
<td></td>
<td>0.63±0.05</td>
<td>0.95±0.07</td>
</tr>
<tr>
<td>Standard drug</td>
<td>10 mg/kg</td>
<td>0.48±0.03*</td>
</tr>
<tr>
<td>Lawsonia inermis Linn</td>
<td>200mg/kg</td>
<td>0.57±0.06</td>
</tr>
<tr>
<td>Chlorophyrum borivilianum Sant</td>
<td>200mg/kg</td>
<td>0.53±0.02</td>
</tr>
<tr>
<td>Combination</td>
<td>200mg/kg</td>
<td>0.53±0.02</td>
</tr>
</tbody>
</table>

Values are mean±S.E.M. (n = 6), Experimental groups were compared with control *(P<0.05), **(P<0.01), ****(P<0.001)

Table 2: Cotton pellet induced granuloma

<table>
<thead>
<tr>
<th>S.no</th>
<th>Treatment</th>
<th>Wt of dry granuloma</th>
<th>%Inhibition of dry granuloma</th>
<th>Wt of wet granuloma</th>
<th>% inhibition of wet granuloma</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>49.17±2.386</td>
<td>---</td>
<td>186.7± 6.146</td>
<td>---</td>
</tr>
<tr>
<td>2</td>
<td>Indomethacin</td>
<td>20.83±1.537***</td>
<td>57.63</td>
<td>92.5±3.819</td>
<td>50.45***</td>
</tr>
<tr>
<td>3</td>
<td>Linermis Linn</td>
<td>22.17±1.138***</td>
<td>54.91</td>
<td>105.0±3.162</td>
<td>43.76***</td>
</tr>
<tr>
<td>4</td>
<td>Chlorovillain</td>
<td>22.67±2.060***</td>
<td>53.89</td>
<td>102.5±2.141</td>
<td>45.09***</td>
</tr>
<tr>
<td>5</td>
<td>Combination</td>
<td>21.83±2.522***</td>
<td>55.6</td>
<td>98.33±5.270</td>
<td>47.33***</td>
</tr>
</tbody>
</table>

Values are mean±S.E.M. (n = 6) Experimental groups were compared with control *(P<0.05), **(P<0.01), ****(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 Chlorophyrum 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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