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Parveen Kumari
S.S.D institute of pharmacy and medical technology, Jhajjar, Haryana 124103, India

Nitu Singh
Department of Pharmacognosy Nims University, Shobha Nagar Jaipur, Rajasthan 303121, India

Deepak Kumar
Vaish institute of pharmaceutical education and science, Rohtak, India

Correspondence:
Parveen Kumari
S.S.D institute of pharmacy and medical technology, Jhajjar, Haryana 124103, India

Synergistic analgesic activity of chloroform extract of *Lawsonia inermis* Linn. and *Chlorophytum borivilianum* Sant.

Parveen Kumari, Nitu Singh, Deepak Kumar

Abstract
This work has done for the determination of the synergistic analgesic activities of chloroform extracts of leaves and roots tubers of *Lawsonia inermis* Linn. and *Chlorophytum borivilianum* Sant. in mice. The chloroform extract was studied for synergistic activity by using tail immersion and hot plate methods. The study revealed that the chloroform extract of both the drugs at the dose level of 200 mg/kg body weight significantly produced analgesic activity, but combination of both the extract showed more analgesic activity as compare to *Lawsonia inermis* Linn and *Chlorophytum borivilianum* Sant. These effect, which may be due to blockade or release of endogenous substances that stimulate pain nerve endings similar to Morphine.

Keywords: *Analgesic, Chlorophytum borivilianum* Sant, *Lawsonia inermis* Linn., *Synergistic*

1. Introduction
Analgesia is an ill-defined, unpleasant sensation, usually evoked by an external and internal noxious stimulus. Analgesics are drugs that selectively relieves pain by acting in the CNS or on peripheral pain mechanisms, without significantly altering consciousness. Analgesics relieve pain nas asymptom, without affecting its cause. Analgesics are divided into two groups, opioid analgesic and non opioid analgesic **[1]** NSAIDS are mainly effective against pain associated with inflammation or tissue damage because they decrease production of the prostaglandins that sensitise nociceptors to inflammatory mediators such as bradykinin [2]. The use of herbal medicines worldwide has provided an excellent opportunity to India to look for therapeutic lead compounds from ourancient system of therapy, i.e. Ayurveda, which can be utilized for development of new drug. Epidemiological evidence suggests that dietary factors play an important role in human health and in the treatment of certain chronic diseases including cancer [3, 4]. In recent years the popularity of complementary medicine has increased. Over 50% of all modern drugs are natural product origin and they play an important role in drug development programs of the pharmaceutical industry [5]. Dietary measures and traditional plant therapies as prescribed by ayurvedic and other indigenous systems of medicine are used commonly in India [6]. Root tubes of *Chlorophytum borivilianum* Sant have been used for a variety of therapeutic purposes such as 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, Herb improves the quantity and flow of breast milk, in feeding mothers. safed musli is all over the world which has made it famous by such names "**Indian Viagra", "Roots of gold", "Herbal Viagra", "The wonder crop"** **[7, 16]**. Leaves of *Lawsonia inermis* Linn Bitter, astringent, acrid, diuretic, emetic, edema, expectorant, anodyne, antiinflammatory, constipating, depurative, liver tonic, haematinic, wound, ulcers, cough, bronchitis, burning sensation, inflammations [17, 20].

2. Materials and methods
2.1 Plant and Preparation of the extracts
The green leaves of *Lawsonia inermis* Linn. and root tubers of *Chlorophytum borivilianum* Sant. were collected locally from the Haryana , India .The plants were identified and authenticated by the botanist ,from NISCAIR.
The tubers and leaves of the both plants were dried under shade and made to a fine powder using a laboratory mill and were extracted with chloroform (Merck specialities Pvt. Ltd,

Mumbai) by using a soxhlet extractor. The phytochemical screening of the chloroform extracts indicated the presence of alkaloids, carbohydrates, tannins and flavonoids in both the extracts.

2.2 Animals
Mice 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 the Institutional Animal Ethics Committee (NU/PH/M/COG /12/78). [21, 22].

2.3 Determination of acute toxicity study
Acute toxicity study of the chloroform extracts showed that the LD 50 value of the both the chloroform extracts on mice was 2000 mg/kg body weight [21, 22]

2.4 Study of the analgesic activity
All the experiments were conducted on an isolated and noiseless condition. The analgesic activity was evaluated by the tail immersion and hot plate method in mice. *Lawsonia inermis* Linn., *Chlorophytum borivilianum* Sant. were dissolved in 1% & 2% gum acacia in distilled water respectively [23, 24].

2.4.1 Tail immersion method
Mice were divided into five groups.
Group 1- Received only water as a control,
Group 2- Treated with morphine 5 mg/kg i.p. as a standard,
Group 3 -Treated with *Lawsonia inermis* Linn. 200 mg/kg p.o.,
Group 4- Treated with *Chlorophytum borivilianum* Sant. 200 mg/kg p.o,
Group 5 -Treated with combination 200 mg/kg p.o.
The distal part of the tails of the animals was immersed in hot water maintained at 55±0.5 ºC. The time taken to withdraw the tail was noted as reaction time. A cut off time 15 sec was maintained at 55 ºC to prevent tissue damaged. The reaction time was measured at 0, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, and 360, 390 min after treatment of standard and extract compounds [25] and result shown in (Table-1& Fig-1)

2.4.2 Hot plate method
Mice were divided into five groups.
Group 1 - Water as a control,
Group 2 - Morphine (sigma) 5 mg/kg i.p. as a standard,
Group 3- Treated with *Lawsonia inermis* Linn. 200 mg/kg p.o.,
Group 4 - Treated with *Chlorophytum borivilianum* Sant. 200 mg/kg p.o,
Group 5 - Treated combination with 200 mg/kg p.o.
The animals were positioned on eddy's hot plate (singhla scientific industries) kept at temperature of 55±0.5 ºC. A cut off period of 15 sec. was observed to avoid damage to the paw. Reaction time was recorded when animals liked their fore or hind paw, or jumped and 0, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 360, 390 min after administration of standardized and extract compounds [26] and result shown in (Table-2 & Fig-2)

2.5 Statistical analysis
All data were expressed as mean±S.E.M, and statistical significance was determined via Dunnett's test by using graph pad prism 5.

3. Result and discussion
The chloroform extract of leaves and root tubers were reduced pain in tail immersion and hot plate analgesic models. The study revealed that the chloroform extract of both the drugs at the dose level of 200 mg/kg body weight significantly produced analgesic activity, but a combination of both the extracts showed more analgesic activity as compared to *Lawsonia inermis* Linn and *Chlorophytum borivilianum* Sant. These effect, which may be due to blockade or the release of endogenous substances that stimulate pain nerve endings similar to Morphine.

4. Acknowledgement
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Table 1: Tail immersion method

Treatment group	0 Min	30 min	60 min	90 min	120 min	150 min	180 min	210 min	240 min	270 min	300 min	330 min	360 min	390 min
Control	2.9± 0.1	2.94± 0.07	2.97±0 .005	2.96± 0.01	2.95± 0.01	2.98± 0.016	2.96± 0.019	2.92± 0.02	2.88± 0.02	2.86± 0.02	2.8± 0.02	2.8± 0.02	2.80± 0.02	2.7± 0.02
standard	2.92± 0.009 ***	4.67± 0.04 ***	6.35± 0.02 ***	8.37± 0.03 ***	9.1± 0.029 ***	12.0± 0.027 ***	12.0± 0.021 ***	11.8± 0.16 ***	11.7± 0.15 ***	11.7± 0.15 ***	11.7± 0.1 ***	11.6± 0.15 ***	1.6± 0.15 ***	11.6± 0.15 ***
L. inermis	2.93± 0.008 ***	3.9± 0.01 ***	4.0± 0.017 ***	5.2± 0.053 ***	6.5± 0.025 ***	7.1± 0.027 ***	7.08± 0.02 ***	7.05± 0.02 ***	7.0± 0.021 ***	6.9± 0.016 ***	6.9± 0.02 ***	6.8± 0.020 ***	6.89± 0.020 ***	6.85± 0.021 ***
C. borivilianum	2.9± 0.01 ***	3.9± 0.011 ***	4.1± 0.02 ***	5.1± 0.024 ***	5.0± 0.022 ***	6.2± 0.018 ***	6.1± 0.019 ***	6.1± 0.02 ***	6.11± 0.01 ***	6.07± 0.02 ***	6.05± 0.02 ***	6.01± 0.01 ***	6.01± 0.019 ***	5.9± 0.019 ***
combination	2.9± 0.008 ***	3.9± 0.010 ***	4.1± 0.019 ***	5.8± 0.020 ***	6.3± 0.017 ***	7.1± 0.028 ***	7.10± 0.021 ***	7.07± 0.022 ***	7.00± 0.029 ***	6.97± 0.028 ***	6.9± 0.032 ***	6.91± 0.03 ***	6.9± 0.030 ***	6.88± 0.032 ***

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

Treatment group	**Table 2:** Hot Plate Method													
	0 Min	30 min	60 min	90 min	120 min	150 min	180 min	210 min	240 min	270 min	300 min	330 min	360 min	390 min
Control	2.3± 0.064	2.42± 0.067	2.47± 0.096	2.48± 0.109	2.51± 0.120	2.50± 0.094	2.48± 0.104	2.44± 0.097	2.42± 0.093	2.42± 0.085	2.39± 0.081	2.40± 0.091	2.36± 0.070	2.37± 0.063
Standard	2.3± 0.099 ***	6.24± 0.113 ***	8.23± 0.099 ***	11.22± 0.089 ***	13.1± 0.048 ***	14.09± 0.099 ***	14.42± 0.128 ***	14.4± 0.124 ***	14.3± 0.126 ***	14.35± 0.1259 ***	14.3± 0.129 ***	14.2± 0.132 ***	14.1± 0.140 ***	14.12± 0.144 ***
L.inermis	2.2± 0.027 ***	3.80± 0.230 ***	4.34± 0.195 ***	4.50± 0.211 ***	4.60± 0.204 ***	4.56± 0.194 ***	4.53± 0.196 ***	4.50± 0.193 ***	4.47± 0.193 ***	4.44± 0.194 ***	4.41± 0.199 ***	4.34± 0.205 ***	4.28± 0.215 ***	4.22± 0.219 ***
Chlorophytum borivilinum	2.3± 0.077 ***	4.01± 0.142 **	4.31± 0.195 ***	4.41± 0.195 ***	4.57± 0.203 ***	4.54± 0.201 ***	4.51± 0.201 ***	4.48± 0.200 ***	4.45± 0.198 ***	4.42± 0.201 ***	4.39± 0.207 ***	4.34± 0.207 ***	4.28± 0.209 ***	4.21± 0.211 ***
Combination	2.3± 0.065 ***	3.82± 0.179 ***	4.05± 0.20 ***	4.31± 0.225 ***	4.44± 0.238 ***	4.37± 0.219 ***	4.38± 0.236 ***	4.32± 0.221 ***	4.29± 0.224 ***	4.26± 0.223 ***	4.23± 0.220 ***	4.17± 0.224 ***	4.09± 0.243 ***	4.09± 0.218 ***

Values are mean ±S.E.M. (n = 6)

Experimental groups were compared with control, *(P<0.05), **(P<0.01), ***(P<0.001)

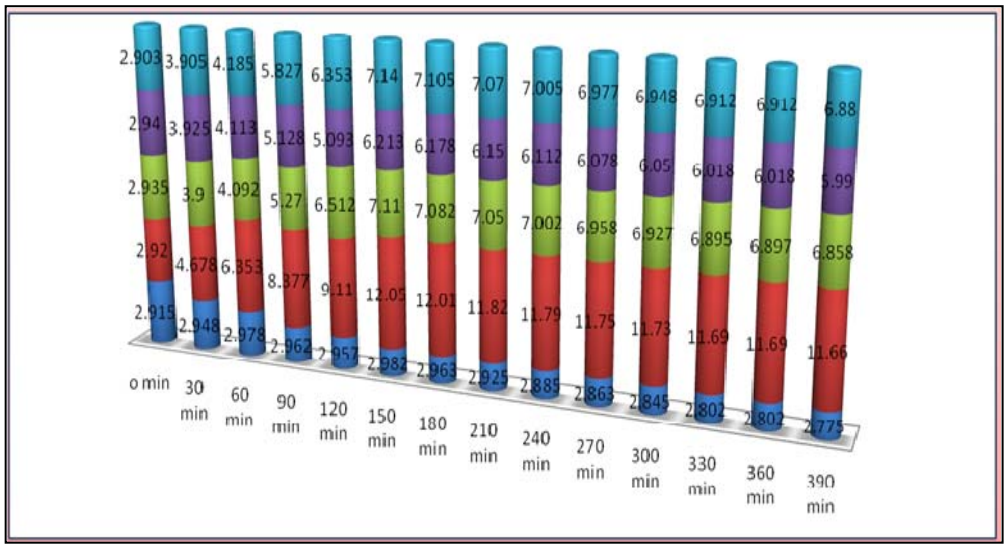

Fig 1: Tail immersion method

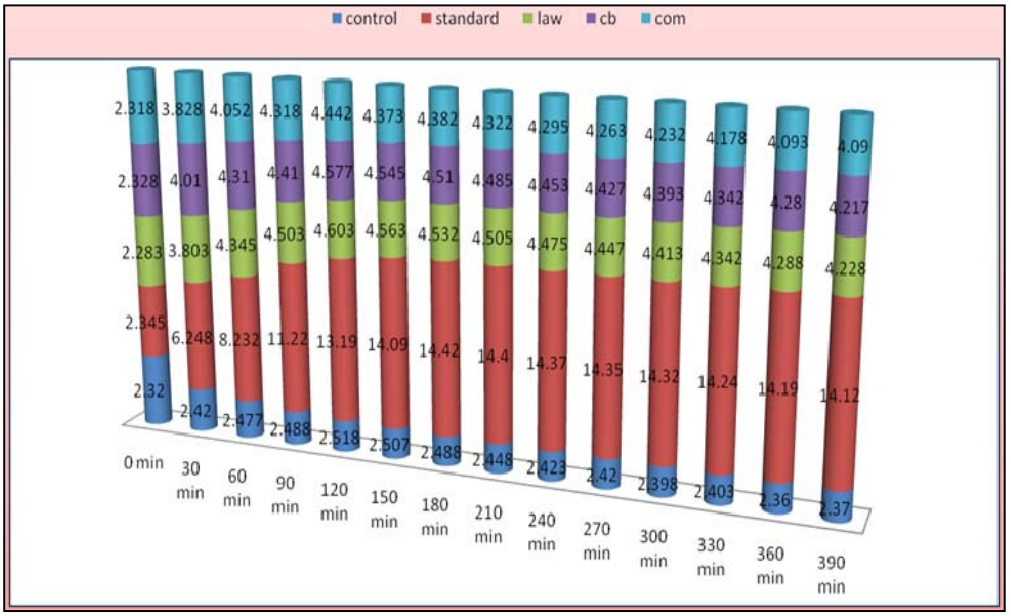

Fig 2: Hot plate method

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