

# Evaluation of Analgesics and Anti-inflammatory Activity of a Poly-Herbal Formulation

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**ABSTRACT:** Rheumatoid arthritis is a chronic multi-systemic disease of unknown cause. It affects the people in their prime of life, predominantly between the ages of 20-50 years with unpredictable course. Various poly-herbal formulations are used in the ayurvedic system of medicine for the treatment of inflammation and pain associated with rheumatoid arthritis, osteo-arthritis, frozen shoulder, ankylosing spondylitis and chronic backache. Our study was aimed to evaluate efficacy of poly-herbal formulation using different animal models such as hot plate reaction time, acetic acid induce writhing in mice, carrageen (1% v/v)-induced paw edema and formaldehyde-induced paw edema. The results indicated that the poly-herbal formulation possesses good analgesic and anti-inflammatory activities in the experimental animal models.

**KEYWORDS:** Analgesic; anti-inflammatory; poly-herbal formulation.

## INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease which is characterized by a series of pathological processes of the joints, such as leukocyte infiltration, pannus formation and extensive destruction of the articular cartilage and bone [1]. RA affects ~1 % of the adult population worldwide [2]. Although there are drugs that have been shown to improve signs and symptoms, alter the natural history of the disease and improve quality of life, but there is still no cure. In addition, these available therapies are associated with potential risks of death or irreversible organ damage [3]. The challenge for society is to balance these known potential risks of therapy with acknowledged benefits despite the fact that these drugs do not lead to a cure. The most commonly prescribed medication for RA treatment is steroidal, non-steroidal anti-inflammatory, disease modifying antirheumatic and immunosuppressant drugs. Though the goal of

these drugs have been to relieve pain and to decrease joint inflammation, to prevent joint destruction and to restore function of disabled joints, these drugs are known to produce various side effects including gastrointestinal disorders, immunodeficiency and humoral disturbances [4]. Accordingly, reducing side effects should be considered while designing improved therapeutics for RA, besides enhancing medicinal effectiveness. The Siddha and Ayurvedic systems of treatment are being increasingly recognized as an alternate approach to arthritic treatment.

*Cissampelos Pereira* Linn. var. *hirsuta* (C.P.) is a very variable, lofty, slender, dioecious, perennial, climber commonly distributed throughout tropical and sub tropical India, ascending up to an altitude of 2,000m, traditionally known as Laghupatha in Ayurveda, an Indian traditional system of medicine [5,6]. The presence of two crystalline alkaloids, hayatin and hayatinin were reported along with the other constituents as quercitol and a sterol. Plant alkaloid has

shown inhibitory activity against human carcinoma cells of the naso-pharynx in cell culture [7]. Effects of hayatin methochloride and (+)-tubocurarine chloride have been studied on autonomic ganglia of cats [8]. Bisbenzylisoquinoline alkaloids which are anti-inflammatory constituents of plants were tested for suppressive effect on *in vitro* nitric oxide (NO) production by lipopolysaccharide-stimulated peritoneal macrophages. The effect was induced with thioglycollate or Bacillus Calmette-Guerin in mice [9]. Plant has been documented for potent diuretic [10], neuromuscular blocking, anti-tumor [11], antibacterial against Gram-positive bacteria [12], anticonvulsants [13] antimalarial [14], antidiarrhoeal [15], antioxidant, antimicrobial and  $\beta$ -glucosidase inhabitation [16], immunomodulatory [17], anti-inflammatory and antiarthritic activities [18].

*Pongamia pinnata* (Linn) Pierre (Leguminosae, Papilionaceae) (P.P.) (synonym: *Pongamia glabra* Vent), popularly known as 'Karanj' or 'Karanja' in Hindi, is a medium sized glabrous tree, found throughout India and further distributed eastwards, mainly in the littoral regions of South Eastern Asia and Australia [19]. This plant indicated the presence of abundant phenylated flavonoids such as furanoflavones, furanoflavonols, chromenoflavones, furanochalcones, and pyranochalcones, flavonoids: Karangin, glabrachalcone, isopongacromene, pongal Pongaglabrone, diketonepongamol, glabrin, karagin, pongapine, kanjone Pongaflavanol and Tunicatachalcone [20-23]. The different parts of this plant have been prescribed for the treatment of various ailments in the ayurvedic literature of India. The seed and seed oil of this plant have been recommended for the treatment of various inflammatory and infectious diseases such as leucoderma, leprosy, lumbago, muscular and articular rheumatism [24]. The leaves are hot, digestive, laxative, anthelmintic and cure piles, wounds and other inflammations [6]. A hot infusion of leaves is used as a medicated bath for relieving rheumatic pains and for cleaning ulcers in gonorrhea and scrofulous enlargement [25]. The different extracts of roots and seeds (ethanol, benzene, petroleum ether) of *Pongamia pinnata* have already been reported to possess anti-inflammatory and antinociceptive activities [26-28]. Also, 70% ethanol extract of *Pongamia pinnata* leaves (PLE) was evaluated for its antiinflammatory activity in rats [29].

*Vitex negundo* Linn. Verbenaceae, (V.N.) known as Nirgundi in Hindi, grows gregariously in wastelands and is also planted as a hedge-plant. It is an erect, 2–5 m in height, slender tree with quadrangular branchlets distributed throughout India. The leaves have five leaflets in a palmately arrangement, which are lanceolate, 4–10 cm long, hairy beneath and pointed at both ends. The bluish purple flowers are numerous

[30]. Among the chemical constituents, it has several flavonoids such as casticin, orientin, isoorientin, luteolin, lutein-7-O-glucoside, corymbosin, gardenins A and B, 3-O-desmethylermetin, 5-O-desmethylnobiletin and 30, 40, 5, 50, 6, 7, 8-heptamethoxyflavone. Besides, many glycosidic iridoids, alkaloids, and terpenoids have also been isolated [31-33]. Plant has been documented for potent antiinflammatory, antipyretic and febrifuge properties [34], are claimed, it has also been investigated for an anti-inflammatory [35, 36], anticonvulsant [37, 38], hepatoprotective [39] and bronchial relaxant [40] actions. Although all parts *Vitex negundo* are used as medicine in the indigenous system of medicine, the leaves are the most potent for medicinal use. It is used for treatment of eye-disease, toothache, inflammation, leucoderma, enlargement of the spleen, skin-ulcers, in catarrhal fever, rheumatoid arthritis, gonorrhoea, and bronchitis. They are also used as tonics, vermifuge, lactagogue, emmenagogue, antibacterial, antipyretic and antihistaminic agents [41-43].

The objective of our study was to evaluate the efficacy of poly-herbal formulation and its individual components by virtue of their analgesic and anti-inflammatory potential in laboratory animals using various animal models.

## MATERIALS AND METHODS

### Plants material:

The Mature fresh leaves of *Vitex negundo* and *pongaamia pinnata* were collected from the local region of Pune and root of *Cissampelos pareira* Linn were purchase from local vendor. The plant materials were identified and authenticated taxonomically (V.No. BAPPI, BAVN1 and BACP1 respectively) at Botanical Survey of India, Pune.

### Preparation of extracts:

The roots of *Cissampelos pareira* were washed, cutted into small pieces, and dried under shade. Coarse powder of the roots was made and extracted by maceration with 50% aqueous alcohol for 72 h at room temperature. Mature fresh leaves of *Vitex negundo* and *pongaamia pinnata* were crushed into powder and extracted by maceration with 50% aqueous alcohol for 72 h at room temperature. The whole extract of individual plants was collected in conical flasks, filtered and the solvents were evaporated to dryness under reduced pressure. The poly-herbal formulation extract was then analyzed by qualitative tests and was found to contain alkaloids, flavonoids, glycosides, sterols and tannins [44].

**Animals:**

Swiss albino mice weighing 20-25 g and Wistar rats weighing 150–180 g of either sex were used for the study. The animals were housed in solid-bottomed polypropylene cages and acclimatized to animal house conditions. The rats were fed with commercial rat's diet and water *ad libitum*. The experiments were designed and conducted in accordance with ethical norms approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPSCEA) and Institutional Animal Ethical Committee (IAEC).

**Preparation of poly-herbal formulation:**

Poly-herbal formulation was prepared according to ED<sub>50</sub> of individual herbs. ED<sub>50</sub> of individual plants was found to be, *Cissampelos pareira* L. (400 mg/kg), *Vitex negundo* L. (500 mg/kg) and *Pongamia pinnata* L (300 mg/kg). The % contents of individual plant extract in poly-herbal formulation were, *Cissampelos pareira* L. (33.33 %), *Vitex negundo* L. (41.66%) and *Pongamia pinnata* L (25%).

**Drugs and dosage:**

The poly-herbal formulation was administered orally at doses of 200 mg/kg, 400mg/kg and 600 mg/kg in the form of suspension prepared in double distilled water containing carboxy methyl cellulose (1%, w/v, CMC). Carrageenan was purchased from Sigma Chemicals (Sigma Chemical Co., St. Louis, MO, USA), Aspirin (Disprin, Reck.Benckier, Mumbai), acetic acid (0.6%) and formaldehyde (4 %) were purchased from local market.

**Acute toxicity study:**

Acute toxicity study was performed in accordance with OECD guidelines 425 [45]. No adverse effect or mortality was detected in albino rats up to 3 gm/kg, *p.o* of poly-herbal formulation during the 24 to 72 hrs observation periods. For this period the rats were continuously observed for 5 hrs for any gross behavioral, neurological or autonomic toxic effect and lethally after 24 to 72 hrs.

**Drug treatment:**

Swiss Albino mice/Wistar rats were divided into eight groups of 5 animals each. Group I served as Control (1% (w/v) CMC in double distilled water, *p.o*). Group II was administered standard drug Aspirin (300 mg/kg, *p.o*). Group III-V served as test groups and treated with poly-herbal formulation (200, 400 and 600 mg/kg, *p.o* in double distilled water containing carboxy methyl cellulose (1%, w/v, CMC) respectively). Group VI-VIII were treated with test drugs as an individual herb extracts (C.P., 400mg/kg, *p.o*; P.P. 300mg/kg, *p.o* and V.N. 500 mg/kg, *p.o*). The

prepared extract was administered once daily for 3 consecutive days for analgesic activity and once daily for 7 consecutive days for anti-inflammatory activity.

**Hot plate reaction time in mice [18]:**

Swiss albino mice were placed on the hot plate (Analgesiometer, Ugo basile Italy) and the time until either licking or jumping occurs was recorded by a stop-watch. A cutoff period of 30 sec was maintained to avoid damage to the paw. The drugs or vehicle were administered orally and the reaction time was observed again at 0, 15, 30, 60 and 120 min after drug administration.

**Acetic acid induced writhing in mice [18, 46]:**

Albino mice of either sex were used for the study. Test animals were administered orally with the drugs 1 hr prior to acetic acid (0.6% v/v in water, 0.1ml/10g, *i.p.*) administration. The mice were placed individually in glass beakers 5 min after acetic acid injection and were then observed for 45 min and the number of writhing was recorded for each animal.

**Carrageenan-induced paw edema [18, 47]:**

The rats were injected with 0.1 ml of carrageen (1% w/v in water) into the sub-plantar area of right hind paw. The drugs were given orally one hour prior to carrageen injection and treatment continued for 7 consecutive days. The volume of rat paw was measured at 7<sup>th</sup> day using plethysmometer (Ugo Basile, Italy) during treatment period. The results were expressed as the mean hind paw swelling as compared with the initial hind paw thickness.

**Formaldehyde-induced paw edema [48, 49]:**

The volume of the hind paw of the animals was measured initially using plethysmometer. After taking the initial reading, 0.1 ml of formaldehyde (2% v/v in water) was injected into sub-plantar aponeurosis of the left hind foot. The drugs were given orally one hour prior to formaldehyde injection and treatment continued for 7 consecutive days. The paw volume was measured at 1, 2, 3, 4, 5, 6 and 7 day after injection.

**Statistical analysis:**

The observations are represented as Mean  $\pm$  S.E.M. The data were processed by one-way analysis of variance (ANOVA) followed by *Dunnnett's* post hoc test. \**P* < 0.05 was considered significant.

**RESULTS****Hot plate reaction time in mice:**

Aspirin produced a significant (*p*<0.01) increase in the reaction time at 15, 30, 60 and 120 min following administration as compared with control. Individual

herb like CP 400 mg/kg, PP 300 mg/kg and VN 500 mg/kg produced a significant ( $p < 0.05$ ) increase in the reaction time at 60 and 120 min as compared with control. The treatment with poly-herbal formulation (400 mg/kg, and 600 mg/kg) produced dose-dependent increase in the reaction time of mice at 60 and 120

min, showing an analgesic effect when compared with control animals. Moreover, the poly-herbal formulation (200, 400 and 600 mg/kg) showed synergistic effect as compared with individual herb like CP 400 mg/kg, PP 300 mg/kg and VN 500 mg/kg at 60 and 120 min (Table 1).

**Table 1. Effect of poly-herbal formulation on Hot plate reaction time in mice**

Treatments	Increase in Reaction Time(Seconds)				
	Time				
	0 min	15min	30 min	60min	120min
Control (5 mg/kg)	11.76 $\pm$ 0.473	12.3 $\pm$ 0.350	12.36 $\pm$ 0.318	12.42 $\pm$ 0.342	12.54 $\pm$ 0.890
Aspirin (300mg/kg)	12.22 $\pm$ 0.383	15.38 $\pm$ 0.474**	24.02 $\pm$ 2.379**	27.38 $\pm$ 1.595**	27.64 $\pm$ 2.689**
PF (200mg/kg)	11.56 $\pm$ 0.555	12.88 $\pm$ 0.335	14.76 $\pm$ 1.429*	19.14 $\pm$ 1.852*	21.36 $\pm$ 2.182*
PF (400mg/kg)	11.92 $\pm$ 0.489	13.73 $\pm$ 0.507	18.76 $\pm$ 1.429*	22.66 $\pm$ 1.45**	22.46 $\pm$ 2.168**
PF (600mg/kg)	12.07 $\pm$ 0.266	14.94 $\pm$ 0.601**	23.92 $\pm$ 1.819**	26.36 $\pm$ 2.068**	24.22 $\pm$ 1.47**
CP (400mg/kg)	11.64 $\pm$ 0.566	13.06 $\pm$ 0.382	15.18 $\pm$ 1.578	18.94 $\pm$ 1.752*	21.44 $\pm$ 1.36*
PP (300mg/kg)	12.34 $\pm$ 0.373	12.92 $\pm$ 0.366	15.2 $\pm$ 1.479	18.98 $\pm$ 1.294*	20.8 $\pm$ 2.437*
VN (500mg/kg)	11.42 $\pm$ 0.462	13.3 $\pm$ 0.472	15.98 $\pm$ 1.571	18.9 $\pm$ 1.893*	20.58 $\pm$ 2.336*

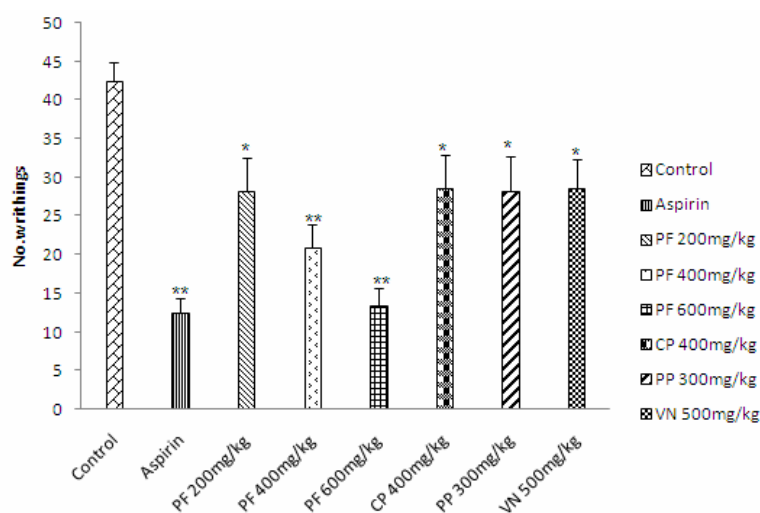
Values are expressed as mean  $\pm$  SEM; n=5. \*  $P < 0.05$ ; \*\*  $P < 0.01$ . CP- *Cissampelos pareira*, PP- *Pongamia pinnata*, VN- *Vitex negundo*.

#### Acetic acid induced writhing in mice:

Aspirin significantly ( $p < 0.01$ ) inhibited the acetic acid induced writhing episode as compared with control. Pretreatment of mice with poly-herbal formulation (200, 400 and 600 mg/kg, *p.o.*) significantly inhibited the acetic acid induced writhing episodes in a dose dependent fashion with an inhibition of 33.5%, 50.48% and 68.4% respectively when compared against control group. Hence, the poly-herbal formulation (200, 400 and 600 mg/kg) showed synergistic inhibition of writhing effect as compared to individual herbs like CP 400 mg/kg, PP 300 mg/kg and VN 500 mg/kg.

#### Carrageenan-induced hind paw edema:

Aspirin significantly ( $p < 0.01$ ) suppressed the paw edema at 2, 3, 4, 5 and 6 hr. The poly-herbal formulation (200 mg, 400 mg and 600 mg/kg) significantly ( $P < 0.05$ ) and dose-dependently reduced the carrageenan-induced paw edema at the 2, 3, 4, 5, and 6 hr when compared with the control. Further, the individual herb (CP 400 mg/kg, PP 300 mg/kg and VN 500 mg/kg) also significantly reduced the carrageenan-induced paw edema at 5 and 6 hr. Overall, the poly-herbal formulation (200, 400 and 600 mg/kg) synergistically inhibited the paw edema as compared to individual herbs at 4, 5 and 6 hr (Table 2).



**Figure 1: Effects of poly-herbal formulation, aspirin and individual herb extracts on writhing test.**

(n=5), Results are expressed as % mean  $\pm$  S.E.M and analysed by one way ANOVA followed by Dunnett's test. \*  $P < 0.05$ , \*\*  $P < 0.01$ . CP- *Cissampelos pareira*, PP- *Pongamia pinnata*, VN- *Vitex negundo*.

**Table 2. Effect of poly-herbal formulation on carrageenan-induced hind paws edema**

Paw edema Volume (ml)							
Treatment	Time						
	0 min	1hr	2hr	3hr	4hr	5hr	6hr
Control	0.498 ± 0.011	0.506 ± 0.012	0.476 ± 0.016	0.472 ± 0.022	0.474 ± 0.024	0.494 ± 0.007	0.482 ± 0.038
ASP	0.426 ± 0.016	0.402 ± 0.025**	0.312 ± 0.010**	0.278 ± 0.016**	0.238 ± 0.024**	0.18 ± 0.034**	0.128 ± 0.021**
PF 400mg/kg	0.464 ± 0.013	0.458 ± 0.013	0.338 ± 0.032*	0.334 ± 0.015**	0.296± 0.033**	0.256± 0.019**	0.218 ± 0.036**
PF 600mg/kg	0.44 ± 0.023	0.404 ± 0.013	0.296 ± 0.021**	0.29 ± 0.020**	0.254 ± 0.026**	0.226 ± 0.028**	0.16 ± 0.044**
CP 400mg/kg	0.478 ± 0.03247	0.466 ± 0.02874	0.472 ± 0.051	0.376 ± 0.025*	0.326 ± 0.040*	0.32 ± 0.028**	0.3 ± 0.035**
PP 300mg/kg	0.452 ± 0.008	0.47 ± 0.020	0.47 ± 0.029	0.386 ± 0.028*	0.332 ± 0.043*	0.326 ± 0.033**	0.292 ± 0.026**
VN 500mg/kg	0.46 ± 0.019	0.436 ± 0.022	0.396 ± 0.029	0.384 ± 0.015*	0.316 ± 0.049*	0.288 ± 0.043**	0.294 ± 0.026**

Values are expressed as Mean  $\pm$  SEM; n =5. \*  $P < 0.05$ ; \*\*  $P < 0.01$ . CP- *Cissampelos pareira*, PP- *Pongamia pinnata*, VN- *Vitex negundo*.

#### Formaldehyde-induced paw edema:

Aspirin produced a significant suppression of paw edema at 2, 3, 4, 5, 6 and 7 day. The poly-herbal formulation (200 mg, 400 mg and 600 mg/kg) produced dose-dependent and significant ( $P < 0.05$ ) reduction in the formaldehyde induced paw edema at the 2, 3, 4, 5, 6 and 7 day when compared against

control. Further, the individual herb (CP 400 mg/kg, PP 300 mg/kg and VN 500 mg/kg) also significantly reduced the formaldehyde-induced paw edema at 4, 5, 6 and 7 day. The poly-herbal formulation produced synergistic inhibition of paw edema as compared to individual herbs at 4, 5, 6 and 7 day.

Table 3. Effect of poly-herbal formulation on Formaldehyde-induced paw edema.

Treatments	Paw Edema volume (ml)											
	Duration											
	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6	Day 7
Control	0.448	±	0.474	±	0.43 ± 0.030		0.428	±	0.408	±	0.404	± 0.424
	0.038		0.015				0.028		0.029		0.026	0.019
Aspirin	0.458	±	0.328	±	0.23	±	0.17	±	0.134	±	0.086	± 0.05
PF	0.030		0.007**		0.016**		0.011**		0.011**		0.012**	0.017**
200mg/kg	0.466	±					0.254	±	0.198	±	0.154	± 0.134
PF	0.042		0.37 ± 0.024		0.3 ± 0.019*		0.019**		0.014**		0.013**	0.021**
400mg/kg	0.472	±	0.348	±	0.226	±	0.19	±	0.162	±	0.134	± 0.104
PF	0.031		0.025*		0.024**		0.018**		0.013**		0.011**	0.018**
600mg/kg	0.482	±	0.334	±	0.214	±	0.178	±	0.148	±	0.122	± 0.076
CP	0.044		0.028**		0.019**		0.013**		0.017**		0.013**	0.012**
400mg/kg	0.458	±	0.372	±	0.294	±	0.256	±	0.19	±	0.166	± 0.154
PP	0.044		0.035		0.024*		0.021**		0.020**		0.019**	0.021**
300mg/kg	0.48	±	0.372	±	0.284	±	0.24	±	0.19	±	0.164	± 0.15
VN	0.011		0.013		0.019*		0.015**		0.008**		0.008**	0.010**
500mg/kg	0.462	±			0.314	±	0.246	±	0.196	±	0.162	± 0.15
	0.053		0.39 ± 0.043		0.038*		0.047**		0.030**		0.021**	0.019**

Values are expressed as Mean ± SEM; n=5. \*  $P < 0.05$ ; \*\*  $P < 0.01$ . CP- *Cissampelos pareira*, PP- *Pongamia pinnata*, VN- *Vitex negundo*.

## DISCUSSION AND CONCLUSION

The results of the present study shows that the Poly-herbal formulation possesses significant anti-inflammatory and analgesic activities in all the tested experimental animal models indicating inhibition of all phases of inflammation. The Poly-herbal formulation possesses central analgesic activity which was evaluated using hot plate method and peripheral activity in acetic acid induced writhing test. Acetic acid causes analgesia by liberating endogenous substances and many others that excite pain at nerve endings [50, 51]. According to the percentage inhibition on the number of writhes obtained with the various doses of the poly-herbal formulation, it was found that the intensity of the analgesic effect was similar to that of aspirin. Aspirin inhibit cyclooxygenase in peripheral tissues, thus interfering with the mechanism of transduction in primary afferent nociceptors [52]. The development of edema in the

paw of the rat after injection of formalin and carrageen is a biphasic event. Inflammation induced by formaldehyde is biphasic, an early neurogenic component is mediated by substance P and bradykinin followed by a tissue mediated response where histamine, 5-HT, prostaglandins and bradykinin are known to be involved [53]. The initial phase of the edema is due to the release of histamine and serotonin and the edema is maintained during the plateau phase by kinin like substance [54] and the second accelerating phase of swelling due to the release of prostaglandin like substances. Hence, it is speculated that apart from inhibition of chemical mediators of inflammation, poly-herbal formulation may also modulate the pain response in the central nervous system.

To conclude, the poly-herbal formulation possesses potent analgesic and anti-inflammatory effects.

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