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Der Pharmacia Lettre, 2010: 2 (1) 285-290
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Comparative evaluation of polyherbal formulations for anti-inflammatory and analgesic activity in rats and mice

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Abstract

Polyherbal formulations (MFT09 and MFG09) containing extracts of various plant constituents viz: *Boswellia serrata, Commiphora wightii, Withania somnifera, Curcuma longa, Tinospora cordifolia, Zingiber officinale, Alpinia galangal, Cyperus rotundus* and *Vitex negundo,* were evaluated for anti-inflammatory and analgesic activity. Anti-inflammatory activity were investigated in albino wistar rats using Carageenan induced hind paw edema model, while the Radiant heat tail flick test was used as a model for evaluating analgesic activity. Treatment with MFT09 resulted in significant decrease in hind paw swelling as compared to MFG09. The effect of MFT09 produced significant analgesic activity against thermal induced pain stimuli in mice at various time intervals post treatment as indicating by increased latency to flick the tail which suggests that its activity might have resulted from its central action. Meanwhile, the results of the acute toxicity test, for oral as well as topical preparation of MFT09 and MFG09 respectively indicate that it is relatively safe and/or non-toxic to rats. The findings of these experimental animal studies indicate that MFT09 possesses potential anti-inflammatory and analgesic activity as compared to MFG09.

Keywords: MFT09, MFG09, Carageenan, anti-inflammatory, analgesic activity.

Introduction

Medicinal herbs as a potential source of therapeutic aids have attained a significant role in health system all over the world for both humans and animals, not only in the diseased condition but also as potential material for maintaining good health [1]. Since time immemorial, medicinal plants, nature's hidden and to a large extent unexplored treasure, have been used virtually in all human cultures around the world (over 75 % of the population) as a source of safe and effective medicines [2].

Inflammation or phlogosis is a pathological response of living tissue to injuries that leads to the local accumulation of plasmatic fluid and blood cells. It is a body defense reaction in

order to eliminate or limit the spread of injurious agent as well as to remove the consequent necrosed cells and tissues [3]. Although it is basically a defense mechanism, the complex events and mediators involved in inflammatory reaction can induce, maintain or aggravate many diseases [4]. Pain is universally understood as a signal of disease and is the most common symptom that brings a patient to a physician's attention, requiring treatment with analgesic agents [5]. Chronic pain is often accompanied by depression [6]. Selective serotonin reuptake inhibitors used in the management of depression, by increasing the serotonin level, inhibit the release of transmitters carrying the pain sensation from the nerve endings.

Currently available remedy for pain and inflammation mainly include Cortico-steroids and Non-steroidal anti-inflammatory drugs for the relief of pain and inflammation. All these therapies are however associated with adverse effects [7]. An investigation on efficacy of plant based drugs used in the traditional medicine has been paid great attention to because they are cheap and have fewer side effects [8]. MFT09 and MFG09 contain extracts of medicinal plants viz *Boswellia serrata, Commiphora wightii, Withania somnifera, Curcuma longa, Tinospora cordifolia, Zingiber officinale, Alpinia galangal, Cyperus rotundus* and *Vitex negundo.* These constituents are used in folk medicine for the treatment of inflammation and pain. The present investigation was undertaken to comparatively evaluate the Polyherbal formulations (MFT09 and MFG09) for possible anti-inflammatory and analgesic activity.

Materials and Methods

Animals

Albino rats of Wistar strain (weighing 100-200 g) and Albino mice of Swiss strain (weighing 25-35 gm) of either sex, obtained from Bharat Serum and Vaccines, Thane, India were housed under standard conditions of temperature ($24+1^0$C), relative humidity (65+10 %), 10-h light and 14-h dark cycle and fed with standard pellet diet (Chakan Mill Ltd, Pune, India) with water *ad libitum*. All the experimental procedures and protocols used in the study were reviewed by the Institutional Animal Ethics Committee (Approval number of project: 080907 and Registration Number of institute: 25/1999/CPCSEA) and were in accordance with the guidelines of the CPCSEA, Ministry of Forests and Environment, Government of India. The animals were deprived of food for 24 hour before experimentation but allowed free access to water throughout.

Drugs and chemicals

Poly Herbal Formulations MFT09 and MFG09 were a gift sample from Om Pharmaceuticals, Bangalore. The dry powder of MFT09 was reconstituted using 0.5% w/v Sodium Carboxy Methyl Cellulose (SCMC) to get 1 mg ml^{-1} of MFT09. The suspension was freshly prepared before use. Carageenan from Sigma Chemical Co, St Louis, MO, USA., Aspirin from Themis Pharmaceuticals, Mumbai., Piroxicam gel from Cipla Limited, Ahmadabad., and all other chemicals, reagents used were of analytical grade.

Acute toxicity studies

For oral preparation (MFT09), Albino wistar rats weighing 100-200 g were divided into two groups of three animals each (three male & three female) the homogenous suspension was prepared freshly, using 0.5% (w/v) Na-Carboxyl Methyl Cellulose (CMC) using a mortar and pestle. The animals were administered 2 g/kg dose p.o. of MFT09 suspension (OECD Guidelines No.423). For topical preparation (MFG09), Albino wistar rats weighing 100-200 g

were divided into two groups of five animals each (five male & five female). The animals were applied 2 g/kg dose topically to 10 percent of the dorsal body surface area of MFG 09 (OECD Guidelines No.402). Then animals were critically observed for clinical symptoms, behavioural changes and mortality up to 72 h period and then upto a period of 14 days.

The studies were carried out by using nine groups of six animals each for both oral preparation (MFT09) & topical preparation (MFG09) in anti-inflammatory (Carageenan induced hind paw edema using albino wistar rats) and analgesic activity (Radiant heat tail flick test using Albino wistar mice).

Group I received normal saline (0.5% Sodium CMC solution) serve as a control,
For oral preparation:- Test drug (MFT09), Group II received MFT09 (200 mg/kg p.o.), Group III received MFT09 (400 mg/kg p.o.), Group IV received MFT09 (600 mg/kg p.o.), Group V received Standard drug (Aspirin 10 mg/kg p.o.),
For topical preparation:- Test drug (MFG09), Group VI received MFG09 (100 mg/kg topically), Group VII received MFG09 (400 mg/kg topically), Group VIII received MFG09 (800 mg/kg topically) and Group IX received Standard drug (Piroxicam gel 10 mg/kg topically).

Anti-inflammatory activity *(Carageenan-induced paw edema)*
Inflammation was induced by a 0.1 ml injection of 1% w/v suspension of carageenan in saline to the plantar surface of right hind paw [9]. For oral preparation, test and standard drugs (MFT09 & Aspirin) were administered orally to the respective groups 60 minutes prior to carageenan injection [10], [11]. For topical preparation, test and standard drugs (MFG09 & Piroxicam gel) were applied to the plantar surface of the hind paw by gently rubbing 50 times with the index finger to the respective groups 60 minutes prior to carageenan injection [12]. The change in the inflammatory reaction was measured using Digital plethysmometer on various time intervals (0, 1,2,3,6 & 24 hr) and compared with control group. The right hind paw edema inhibition at different doses of Test drug and Standard drug were calculated by comparing with vehicle treated control rats.

Following formula was used:

$$\textbf{\% inhibition of paw edema} = \frac{(Vt\text{-}Vo)_{control} - (Vt\text{-}Vo)_{treated}}{(Vt\text{-}Vo)_{control}} \times 100$$

Where,
Vt is the rat paw volume at time‘t’, Vo is the initial rat paw volume (before Carageenan injection), $(Vt\text{-}Vo)_{control}$ is edema produced in control group and $(Vt\text{-}Vo)_{treated}$ is edema produced in treatment groups.

Analgesic activity (*Radiant heat tail flick test*)
A radiant heat tail flick analgesiometer was used to measure response latencies. For oral preparation, test and standard drugs (MFT09 & Aspirin) were administered orally to the respective groups 30 minutes before taking response [13]. For topical preparation, test and standard drugs (MFG09 & Piroxicam gel) were applied topically to the tail of respective groups 30 minutes before taking response [14]. Basal reaction time of all the albino wistar mice to radiant heat was recorded by placing the tail (1.5 cm measured from the root of the tail) on the radiant heat source. The cut-off reaction is fixed at 15 sec to avoid tissue damage. The tail withdrawal (flicking action) from the heat source i.e, reaction time was recorded at 0 min, 30 min, 1 hr, 2 hr & 4 hr.

Statistical analysis

All the values are expressed as mean ± S.E.M. The results were analyzed statistically by Analysis of Variance (ANOVA) followed by Dunnett's test. *P* values <0.05 were considered significant.

Results and Discussion

In the preliminary acute toxicity study, MFT09 & MFG09 seems to be safe up to 2 g/kg because even at this high dose no toxic or deleterious effects were observed immediately or during 3 days and upto 14 days of observation period.

Anti-inflammatory activity

The anti-inflammatory effects of the MFT09 & MFG09 are shown in Table-1. The MFT09 (200,400 and 600 mg/kg, p.o.) showed very significant results and caused an inhibition in the carageenan-induced hind paw edema in rats compare to MFG09 (100, 400 and 800 mg/kg, topically). The MFT09 showed dose dependent inhibition of paw edema in the first and second phase as compared to MFG09.

Table 1: Effect of MFT09 & MFG09 in Carageenan induced hind paw edema model

Treatment and dose (mg/kg) p.o.	Paw volume (ml)					
	0hr	1hr	2hr	3hr	6hr	24hr
Group I Control	0.84 ±0.007	1.07 ±0.03	1.30 ±0.01	1.49 ±0.03	1.36 ±0.04	0.92 ±0.01
Group II MFT09 (200)	0.86 ±0.008	1.04 ±0.01 (21.73)	1.26 ±0.009 (13.04)	1.28 ±0.031** (35.4)	1.16 ±0.028** (42.3)	0.91 ±0.02 (37.5)
Group III MFT09 (400)	0.84 ±0.012	1.14 ±0.028 (-30.4)	1.22 ±0.023 (16.3)	1.22 ±0.021** (41.5)	1.07 ±0.028** (55.7)	0.90 ±0.014 (25)
Group IV MFT09 (600)	0.87 ±0.01	1.04 ±0.017 (21.73)	1.18 ±0.02** (32.6)	1.26 ±0.02** (40)	1.03 ±0.015** (69.2)	0.91 ±0.023 (50)
Group V Aspirin (10)	0.87 ±0.015	1.12 ±0.018 (-8.69)	1.20 ±0.029* (28.2)	1.12 ±0.02** (61.5)	0.99 ±0.01** (76.9)	0.87 ±0.019 (75)
Group VI MFG09 (100)	0.88 ±0.009	1.31 ±0.01 (-0.15)	1.36 ±0.02 (0.43)	1.37 ±0.01 (1.7)	1.32 ±0.01 (1)	1.0 ±0.02 (0.49)
Group VII MFG09 (400)	0.89 ±0.016	1.3 ±0.017 (0.76)	1.35 ±0.025 (1.16)	1.35 ±0.03 (3.1)	1.3 ±0.01 (3)	0.96 ±0.02 (4.4)
Group VIII MFG09 (800)	0.87 ±0.01	1.30 ±0.01 (-0.53)	1.33 ±0.01 (0.07)	1.28 ±0.02 (6.5)	1.21 ±0.03 (5.5)	0.93 ±0.02 (5.2)
Group IX Piroxicam gel (10)	0.86 ±0.01	1.30 ±0.009 (0.38)	1.33 ±0.01 (2.5)	1.28 ±0.02* (8.3)	1.21 ±0.03** (9.3)	0.93 ±0.02 (9.1)

MFT09-Test drug, *N* = 6 in each group, Values are mean ± S.E.M, one way ANOVA followed by Dunnet's test, * *P*< 0.05 vs Arthritic control, ** *P*< 0.01 vs arthritic control. Values in the bracket indicate percent inhibition.

Analgesic activity

In Radiant heat tail flick test, Treatment with MFT09 (200,400 and 600 mg/kg, p.o.) produced significant analgesic action against thermal induced pain stimuli in albino wistar mice at various time points post treatment by increasing latency to flick the tail as compared to MFG09 (100, 400 and 800 mg/kg, topically) (TABLE-2).

Table 2: Effect of MFT09 & MFG09 on reaction time in Radiant heat tail flick method

Treatment and dose (mg/kg)	Pre-drug (mean ±SEM) reaction time (in second)	Reaction time in sec (mean ± SEM)			
		30 min	1 hour	2 hour	4 hour
Group I Control	4.438± 0.18	4.215±0.09	4.14±0.16	4.043±0.08	3.973±0.058
Group II MFT09 (200)	4.373± 0.18	4.918±0.27*	5.933±0.26**	6.97±0.06**	6.905±0.24**
Group III MFT09 (400)	3.757±0.20	5.31±0.16**	6.79±0.29**	7.68±0.21**	7.888±0.35**
Group IV MFT09 (600)	4.323±0.19	6.192±0.18**	7.378±0.14**	8.95±0.18**	8.847±0.21**
Group V Aspirin (10)	3.918±0.17	6.94±0.08**	7.562±0.15**	8.98±0.073**	8.735±0.09**
Group VI MFG09 (100)	4.01±0.206	3.74± 0.232	3.642±0.199	3.60±0.245	3.738±0.190
Group VII MFG09 (400)	4.02±0.221	3.795± 0.210	3.942±0.128	4.18±0.172	3.995±0.179
Group VIII MFG09 (800)	3.71±0.238	3.267± 0.156	3.037±0.289	3.59±0.258	3.555±0.258
Group IX Piroxicam gel(10)	3.66±0.248	3.313± 0.264	3.647±0.206	4.91±0.282*	4.603±0.151*

N = 6; Each data suggest Mean ± SEM, One-way ANOVA followed by Dunnett's test is applied for statistical analysis, Drug treated groups were compared with control group. ** Significant at $p < 0.01$, * Significant at $p < 0.05$

The most widely used primary test for screening of anti-inflammatory agents is carageenan induced edema in rat hind paw [15]. The development of the paw edema in the rats after the injection of carageenan has been described as a biphasic event, the first phase is due to release of histamine and serotonin (5-HT) (1 hr), first plateau phase is maintained by kinin like substance (2hr) and second accelerating phase of swelling is attributed to Prostaglandin release (3hr) [16]. Histamine and 5-HT are mainly responsible for vasodilatation and increased vascular permeability. Kinins, once released, are able to activate B_1 and/or B_2 receptors, releasing other inflammatory mediators, such as Prostaglandins (PGs), Leukotrienes (LTs), Histamine, Nitric oxide (NO), platelet activating factor (PAF) and

cytokines, among others derived mainly from leucocytes, mast cells, macrophages and endothelial cells, causing either cell influx and plasma extravasations [17]. However the effect of MFT09 was more significant in the second phase and maximum inhibition was observed at 3rd hour after carageenan injection compare to MFG09. The effect could be co-related to the inhibitory action of MFT09 on release of kinins and inhibition of prostaglandin synthesis.

The Radiant heat tail flick method has been found to be suitable for the evaluation of centrally acting analgesics. It involves higher brain functions and consists of responses to nociceptive stimuli organized at a supraspinal level [18]. Thus based on the ability of MFT09 to attenuate the thermally induced nociception, it is plausible to suggest that MFT09 is exhibiting its effect by a central mechanism.

Conclusion

It can be concluded that MFT09, a Polyherbal formulation possesses potential anti-inflammatory and centrally acting analgesic activity as compared to MFG09.

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