OPEM
www.opem.org

Oriental Pharmacy and Experimental Medicine 2009 **9(3)**, 232-237
DOI 10.3742/OPEM.2009.9.3.232

Analgesic and anti-inflammatory activity of a polyherbal formulation (PHF-AROGH)

M Mohan[1,*], VS Gulecha[1], VM Aurangabadkar[1], R Balaraman[2], A Austin[3] and S Thirugnanasampathan[3]

[1]Department of Pharmacology, M. G. V's College of Pharmacy, Panchavati, Nasik, Maharashtra 422003, India; [2]Department of Pharmacy, Faculty of Technology and Engineering. The M. S. University of Baroda, Kalabhavan, Baroda, Gujarat, 390001, India; [3]Rumi Herbals Pvt. Ltd., B-25, JJ Nagar, Mugappair West, Chennai-600 037, India

Received for publication May 21, 2008; accepted March 4, 2009

SUMMARY

The effect of arogh, a polyherbal formulation-PHF [each 3 g powder contained Nelumbo nucifera G. (0.24 g), Hemidesmus indicus R. (0.24 g), Zingiber officinale R. (0.24 g), Terminalia chebula R. (0.24 g), Quercus infectoria O. (0.12 g), Hibiscus rosa-sinensis L. (0.24 g), Rosa damascene M.(0.24 g), Eclipta alba H.(0.24 g), Glycyrrhiza glabra L. (0.24 g)] was investigated in various experimental models of pain and inflammation. Analgesic activity of PHF was studied in mice using acetic acid induced writhing, tail immersion and hot plate methods. Anti-inflammatory activity of PHF was studied in rats using carrageenan induced hind paw edema and formalin induced rat paw edema methods. PHF significantly ($P < 0.05$) reduced the number of writhings, increased latency to flick tail in tail immersion method and elevated the mean basal reaction time in hot plate method. PHF significantly ($P < 0.05$) inhibited carrageenan induced hind paw edema and formalin induced rat paw edema. The PHF was tested at dose of 30, 100, 300 and 500 mg/kg.

Key words: PHF; Analgesic; Anti-inflammatory; Acetic acid; Carrageenan; Formalin

INTRODUCTION

Since time immemorial, indigenous plants have been a major source of medicine. In folk medicine, they are used, in single or in combined forms for treating different types of inflammatory and arthritic conditions. Prolonged administrations of steroidal and nonsteroidal anti-inflammatory drugs are known to be associated for their adverse effects. Herbal drugs have lesser side effects and are largely replaced by synthetic drugs. Arogh, a polyherbal ayurvedic formulation, composed of 9 plant ingredients was tried for its analgesic and antiinflammatory activities. Some of its constituents like E. alba (Karthikumar *et al*., 2007), Z. officinale (Vendruscolo *et al*., 2006), G. glabra (Khaksa *et al*., 1996), T. chebula (Dongmo Bertrand *et al*., 2006), were claimed to reduce inflammation and pain. Arogh has antioxidant property (Suchalatha *et al*., 2004) and is used in myocardial infarction (Suchalatha and Shyamala Devi, 2004). There are no reports on its analgesic and anti-inflammatory activity. The present study is therefore an attempt to assess the PHF for its analgesic and anti-inflammatory activities using various animal models.

*Correspondence: M Mohan, Department of Pharmacology, M. G. V.'s College of Pharmacy, Panchavati, Nasik, Maharashtra, 422003, India. Tel: +919823234514; Fax: +9102532511931; E-mail: mm_nasik@yahoo.co.in

MATERIALS AND METHODS

Chemicals and drugs

Aspirin (Research Lab, Mumbai), Pentazocine (Fortwin, Ranbaxy), Carrageenan (Sigma, Mumbai) and Formalin (Research Lab, Mumbai) were used in the study. PHF-Arogh (Rumi Herbals, Chennai) was a powder formulation. PHF (30, 100, 300 and 500 mg/kg), and Aspirin were prepared in 2% gum acacia suspension before oral administration. Formalin and Pentazocine were dissolved in water for injection before intraperitoneal administration.

Animals

Albino mice (20 - 25 g) and wistar rats (100 - 150 g) were obtained from serum institute, pune. Animals were housed in groups of five at an ambient temperature of 25 ± 1°C. Animals had free access to food and water. Animals were deprived of food but not water 4 h before the experiment. The institutional animal ethical committee approved the protocol of this study.

Acetic acid induced writhing method

In this method, mice in groups of five each were treated with vehicle, PHF (30, 100, 300 and 500 mg/kg). Analgesic activity of PHF (30, 100, 300 and 500 mg/kg) was assessed by counting the number of writhes induced by 0.6% acetic acid (10 ml/kg, i.p.) (Koster and Anderson, 1959; Turner, 1971). Number of writhes per animal was counted in the following 20 min. Aspirin (20 mg/kg, p.o.) was used as a positive standard. PHF and aspirin were administered 1 h prior to intra-peritoneal administration of 0.6% acetic acid. Percentage protection against writhing was taken as an index of analgesia.

It is calculated as:
Number of writhing in control group –Number of writhing in treated group/ Number of writhing in control group × 100

Tail immersion method

Mice in groups of five each were treated with vehicle, pentazocine (17.5 mg/kg, i.p.) and PHF (30, 100, 300 and 500 mg/kg) The distal 2 - 3 cm portion of mouse-tail was immersed in hot water maintained at 55 ± 0.5°C (Turner, 1971). The time taken by the mouse to withdraw the tail from hot water was noted as reaction time.

Hot plate method

Mice in groups of five each were treated with vehicle, pentazocine (17.5 mg/kg, i.p.) and PHF (30, 100, 300 and 500 mg/kg, p.o.) They were placed on a hot plate maintained at a temperature of 55 ± 0.5°C (Turner, 1971). The latency to lick the paw or jump from the hot plate was noted as the reaction time. The reaction time was noted at 0, 15, 30, 45, 60, 90 and 120 min.

The cut off time was considered as 15 s. The cut off time is determined by taking the average reaction time plus 3 times the standard deviation of the combined latencies of the control mice at all time periods.

Carrageenan induced rat paw edema

The method of Winter *et al*. (1962) was used to study acute inflammation. Rats in groups of five each were treated with vehicle, PHF (30, 100, 300 and 500 mg/kg, p.o.) and Aspirin (20 mg/kg) one hour prior to Carrageenan injection. 0.1 ml of 1% Carrageenan was injected into the subplantar tissue of left hind paw of each rat. Swelling of carrageenan injected foot was measured at 0, 1, 2, 3, 4 h using Plethysmometer (UGO Basile, Italy) (Vogel, 2002). The right hind paw was injected with 0.1 ml of vehicle.

Formalin induced rat paw edema

Rats in groups of five each were treated with vehicle, PHF (30, 100, 300 and 500 mg/kg, p.o.), and Aspirin (20 mg/kg) one hour prior to formalin injection. 0.05 ml of 1%w/v solution of formalin was injected into the subplantar tissue of left hind

paw of each rat. Swelling of formalin-injected foot was measured at 0, 1, 2, 3, 4 h using plethysmometer (UGO Basile, Italy) (Roy *et al*., 1982; Dimo *et al*., 2006). The right hind paw was injected with 0.1 ml of vehicle. The % decrease in paw volume is calculated as:

Change in volume in control group - Change in volume in treated group/Change in volume in control group × 100

Statistics

All values are shown as mean ± S.E.M. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's test. $P < 0.05$ was considered statistically significant.

RESULTS

Acetic acid induced writhing method

PHF (30, 100, 300 and 500 mg/kg.) significantly ($P < 0.05$) reduced the number of writhing induced by acetic acid. Maximum percentage of inhibition of writhing response was observed with PHF (500 mg/kg).Aspirin showed a maximum inhibition of writhing response as 49.35%. The observations are given in Table 1.

Tail immersion method

PHF (100, 300 and 500 mg/kg) significantly *(P <* 0.05) increased latency to flick tail as compared to control animals. The highest nociception inhibition was exhibited by PHF (500 mg/kg) at 15 min. The maximum nociception inhibition by pentazocine was observed at 15 min. PHF (30 mg/kg) did not produce any significant nociception inhibition as compared to control group. The observations are given in Table 2.

Table 1. Effect of PHF (30, 100, 300, and 500 mg/kg) on acetic acid induced writhing in mice

Treatment (mg/kg)	Number of writhings	% Inhibition
Control	31.2 ± 0.86	-
Aspirin (20)	15.8 ± 0.91*	49.35
PHF (30)	27.6 ± 0.6*	11.53
PHF (100)	23.4 ± 0.50*	25
PHF (300)	20.8 ± 0.37*	33.33
PHF (500)	15.8 ± 0.86*	49.35
F (5,24); P-value	76.03 0.000	

n = 5. The observations are mean ± S.E.M. * $P < 0.05$, as compared to control. (ANOVA followed by Dunnett's test). PHF = Polyherbal formulation.

Hot plate method

PHF (100, 300 and 500 mg/kg) significantly ($P < 0.05$) elevated the mean basal reaction time as compared to control group. The highest nociception inhibition was exhibited by PHF (500 mg/kg) at 15 min. The maximum nociception inhibition by pentazocine

Table 2. Effect of PHF (30, 100, 300 and 500 mg/kg) on Tail immersion method in mice

Treatment (mg/kg)	Latency to flick tail (sec)						
	0 min	15 min	30 min	45 min	60 min	90 min	120 min
Control	2.8 ± 0.47	2.83 ± 0.47	2.83 ± 0.49	2.83 ± 0.46	2.85 ± 0.47	2.83 ± 0.47	2.85 ± 0.47
Pentazocine (17.5)	2.76 ± 0.25	6.58 ± 0.17*	6.30 ± 0.15*	5.68 ± 0.14*	5.40 ± 0.10*	4.62 ± 0.15*	4.35 ± 0.15
PHF (30)	2.87 ± 0.40	2.91 ± 0.40	2.91 ± 0.41	2.90 ± 0.41	2.89 ± 0.41	2.89 ± 0.41	2.88 ± 0.40
PHF (100)	2.58 ± 0.30	7.03 ± 0.60*	5.86 ± 0.71*	5.23 ± 0.46*	4.91 ± 0.34	4.18 ± 0.29	3.90 ± 0.26
PHF (300)	2.63 ± 0.23	7.78 ± 0.24*	7.21 ± 0.15*	5.44 ± 0.37*	4.66 ± 0.46	3.67 ± 0.38	3.09 ± 0.32
PHF (500)	2.31 ± 0.19	8.01 ± 1.15*	7.53 ± 1.51*	6.52 ± 1.17*	5.62 ± 1.08*	4.61 ± 0.74*	3.57 ± 0.73
F (5, 24)	0.39	10.72	7.99	6.60	4.73	3.24	1.98
	0.849	0.000	0.000	0.000	0.004	0.021	0.116

n = 5.The observations are mean ± S.E.M. * $P < 0.05$, as compared to control. (ANOVA followed by Dunnett's test). PHF = Polyherbal formulation.

Table 3. Effect of PHF (30, 100, 300 and 500 mg/kg) on Hot plate method in mice

Treatment (mg/kg)	Basal reaction time (sec)						
	0 min	15 min	30 min	45 min	60 min	90 min	120 min
Control	2 ± 0.46	1.99 ± 0.46	1.99 ± 0.46	1.99 ± 0.46	1.99 ± 0.46	1.99 ± 0.46	1.99 ± 0.46
Pentazocine (17.5)	2.85 ± 0.33	8.75 ± 0.45*	7.36 ± 0.42*	6.51 ± 0.37*	5.87 ± 0.44*	5.40 ± 0.41*	4.89 ± 0.35*
PHF (30)	2.64 ± 0.51	2.63 ± 0.51	2.64 ± 0.51	2.62 ± 0.54	2.62 ± 0.52	2.61 ± 0.52	2.61 ± 0.52
PHF (100)	2.74 ± 0.28	9.36 ± 1.15*	6.81 ± 0.61*	5.41 ± 0.36*	4.88 ± 0.53*	3.90 ± 0.39*	3.28 ± 0.31
PHF (300)	2.57 ± 0.20	9.40 ± 0.89*	7.82 ± 0.46*	5.16 ± 0.34*	4.41 ± 0.18*	3.78 ± 0.26*	3.15 ± 0.29
PHF (500)	2.38 ± 0.18	10.25 ± 0.61*	9.17 ± 0.62*	8.14 ± 0.52*	7.28 ± 0.33*	5.91 ± 0.22*	4.87 ± 0.17*
F (5, 24)	0.74	25.80	30.94	24.29	21.16	14.87	9.70
P-value	0.587	0.000	0.000	0.000	0.000	0.000	0.000

n = 5.The observations are mean ± S.E.M. *P < 0.05, as compared to control. (ANOVA followed by Dunnett's test). PHF = Polyherbal formulation.

Table 4. Effect of PHF (30, 100, 300 and 500 mg/kg) on Carrageenan Induced Rat Paw Edema

Treatment (mg/kg)	Mean increase in paw volume (ml)					% Inhibition at 4 h
	0 h	1 h	2 h	3 h	4 h	
Control	0.77 ± 0.04	1.16 ± 0.01	1.91 ± 0.01	2.12 ± 0.03	2.18 ± 0.01	-
Aspirin (20)	074 ± 0.06	1 ± 0.03*	1.08 ± 0.03*	1.1 ± 0.03*	1.1 ± 0.03*	49.54
PHF (30)	0.72 ± 0.07	0.97 ± 0.06*	1.34 ± 0.07*	1.4 ± 0.01*	1.39 ± 0.08*	36.23
PHF (100)	0.75 ± 0.05	1 ± 0.01*	1.44 ± 0.08*	1.5 ± 0.05*	1.30 ± 0.06*	40.36
PHF (300)	0.81 ± 0.08	0.99 ± 0.01*	1.51 ± 0.06*	1.6 ± 0.07*	1.30 ± 0.04*	40.33
PHF (500)	0.71 ± 0.06	0.96 ± 0.04*	1.16 ± 0.06*	1.3 ± 0.05*	1.25 ± 0.05*	42.66
F (5,24)	0.28	3.92	21.65	27.26	58.70	
P-value	0.877	0.003	0.000	0.000	0.000	

n = 5. The observations are mean ± S.E.M. *P < 0.05, as compared to control. (ANOVA followed by Dunnett's test). PHF = Polyherbal formulation.

Table 5. Effect of PHF (30, 100, 300, and 500 mg/kg) on Formalin Induced Rat Paw Edema

Treatment (mg/kg)	Mean increase in paw volume (ml)					% Inhibition at 4 h
	0 h	1 h	2 h	3 h	4 h	
Control	0.53 ± 0.01	1.02 ± 0.01	1.16 ± 0.03	1.28 ± 0.03	1.35 ± 0.03	-
Aspirin (20)	0.57 ± 0.01	0.87 ± 0.02*	0.98 ± 0.02*	1.09 ± 0.02*	0.87 ± 0.01*	35.50
PHF (30)	0.59 ± 0.01	0.77 ± 0.01*	0.95 ± 0.01*	1.04 ± 0.01*	0.88 ± 0.02*	34.81
PHF (100)	0.52 ± 0.02	0.75 ± 0.03*	0.93 ± 0.03*	1.01 ± 0.01*	0.85 ± 0.01*	37.03
PHF (300)	0.51 ± 0.02	0.73 ± 0.03*	0.92 ± 0.02*	1.02 ± 0.01*	0.85 ± 0.03*	37.03
PHF (500)	0.50 ± 0.01	0.75 ± 0.02*	0.86 ± 0.02*	0.94 ± 0.01*	0.77 ± 0.02*	42.96
F (5,24)	2.97	26.89	20.41	48.09	94.66	
P-value	0.000	0.000	0.000	0.000	0.000	

n = 5.The observations are mean ± S.E.M. *P < 0.05, as compared to control. (ANOVA followed by Dunnett's test). PHF = Polyherbal formulation.

was observed at 15 min. PHF (30 mg/kg) did not produce any significant nociception inhibition as compared to control group. The observations are given in Table 3.

Carrageenan induced rat paw edema

The PHF (30, 100, 300 and 500 mg/kg) significantly (P < 0.05) inhibited carrageenan induced rat paw edema as compared to control group. Maximum

inhibition of paw edema was observed with PHF (500 mg/kg) at 4 h when compared to the control group. Aspirin inhibited paw edema by 49.54%. The observations are given in Table 4.

Formalin induced rat paw edema

The PHF (30, 100, 300 and 500 mg/kg) significantly ($P < 0.05$) inhibited formalin induced rat paw edema. Maximum inhibition of paw edema was observed with PHF (500 mg/kg) at 4 h when compared to the control group. Aspirin inhibited paw edema by 35.50%. The observations are given in Table 5.

DISCUSSION

The preliminary phytochemical screening of PHF showed the presence of alkaloids, saponins, steroids, flavonoids, and tannins in our laboratory (Kokate, 1994). These compounds have well known anti-inflammatory effects (Capasso *et al.,* 1983; Senatore *et al.,* 1989; Santos *et al.,* 1995; Park *et al.,* 2001). The effects observed with PHF could possibly be due to the synergistic actions of these compounds. In the present study, PHF demonstrated a significant ($P < 0.05$) analgesic and anti-inflammatory activity at different dose levels in various animal models of pain and inflammation. Acetic acid induced writhing is a sensitive method for screening peripheral analgesic effect of compounds. It causes an increase in concentration of PGE2 and PGF2a in the peritoneal fluid (Collier *et al.,* 1968; Bentley *et al.,* 1983). The hot plate method and tail flick method originally described by Woolfe and Mac Donald (1994) has been found to be suitable for the evaluation of centrally but not peripherally acting analgesics. The nociceptors seem to be sensitized by sensory nerves. The involvement of endogenous substances such as PGs may be minimized in this model. In our study, PHF (100, 300 and 500 mg/kg) exhibited a significant analgesic effect in all above models of pain.

Edema induced by phlogistic agents is a widely accepted model for the evaluation of anti-edemal effect of drugs (Winter *et al.,* 1962; El-Shenawy *et al.,* 2002). To assess the anti-inflammatory activity, PHF was evaluated by two popular screening models widely used for NSAID namely Carrageenan induced rat paw edema and Formalin-induced paw edema. Carrageenan induced rat paw edema shows a biphasic effect (Vinegar *et al.,* 1969). The first phase is due to release of histamine and serotonin (5-HT) (0 - 2 h), plateau phase is maintained by kinin like substance (3 h) and second accelerating phase of swelling is attributed to PG release (4 h). In our study, PHF (30, 100, 300 and 500 mg/kg, p.o.) significantly ($P < 0.05$) reduced the edema induced by carrageenan in all three phases.

Formalin induced edema also shows a biphasic response and originate mainly from neurogenic inflammation followed by participation of kinins and leukocytes with their pro-inflammatory factors including PGs (Wheelar-Aceto and Cowan, 1991). According to Chen *et al.*, 1995 acute inflammation induced by formalin results from cell damage which provides the production of endogenous mediators like histamine and bradykinin. Edema produced by formalin was significantly ($P < 0.05$) inhibited by PHF (30, 100, 300 and 500 mg/kg, p.o.). Carrageenan was found to be more potent in inducing edema than formalin, indicating a more reliable model for inflammation. Thus, it is concluded that PHF posses analgesic and anti-inflammatory properties which are probably mediated via inhibition of prostaglandin synthesis as well as central inhibitory mechanism and may have a potential benefit for the management of pain and inflammatory disorders.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. S. B. Kasture and Dr. V. S. Kasture for their continuous support and encouragement.

REFERENCES

Bentley GA, Newton SH, Starr J. (1983) Studies on the

anti-nociceptive action of drugs and their interaction with opioid mechanism against. *Br. J. Pharmacol*. **79**, 125-134.

Chen Y, Tsai H, Wu T. (1995) Anti-inflammatory and analgesic activities form roots of Angelica pubescens. Planta Med. 61, 2-8.

Collier HO, Dinneen LC, Johnson CA, Schneider C. (1968) The abdominal constriction response and its suppression by analgesic drugs in mouse. *Br. J. Pharmacol*. **32**, 295-310.

Capasso F, Cerri R, Morrica P, Senatore F. (1983) Chemical composition and anti-inflamatory activity of an alcoholic extract of Teucrium polium L. *Boll. Soc. Ital. Biol Sper.* **59**, 1639-1643.

Dimo T, Agathe L, Fotio T, Nguelefack B, Asongalem EA, Kamtchouing P. (2006) Anti-inflammatory activity of leaf extracts of Kalanchoe crenata A. *Indian J. Pharmacol*. **38**, 115-119.

Dongmo B, Beppel G, Nole T, Kamayani A. (2006) Analgesic activities of the stem bark extract of Terminalia superba E. (Combrataceae). *Pharmacologyonline*. **2**, 171-177.

El-Shenawy SM, Abdel-Salam OM, Baiuomy AR, El-Baeran S, Arbid MS. (2002) Studies on the anti-inflammatory and antinociceptive effects of Melatonin in rat. *Pharmacol. Res*. **46**, 235-243.

Karthikumar S, Vigneshwari K, Jegatheesan K. (2007) Screening of antibacterial and antioxidant activities of leaves of Eclipta prostrate L. *Scien Res and Essay*. **2**, 101-104.

Khaksa G, Zoltaghari ME, Dehpour AR, Samadian T. (1996) Anti inflammatory and anti nociceptive activity of disodium glycyrhetinic acid and hemipthalate. *Planta Medica*. **62**, 326-328.

Kokate CK. (1994) Practical Pharmacognosy, 3rd Edn., Vallabh Prakashan, New Delhi. 107-109.

Koster R, Anderson M, Beer EJ. (1959) Acetic acid for analgesic screening. Proc. *Soc. Exp. Biol*. **18**, 412-415.

Park EH, Kahng JH, Lee SH, Shin KH. (2001) An anti-inflammatory principle from cactus. *Fitoterapia.* **72**, 288-290.

Roy A, Gupta JK, Lahiri SC. (1982) Further studies on anti-inflammatory activity of two potent indan-1-acetic acids. *Indian J. Physiol. Pharmacol*. **26**, 206-214.

Santos AR, Niero R, Filho VC, Yunes RA, Pizzolatti MG, Delle Monache F, et al. (1995) Anti-nociceptive properties of steroids isolated from Hyllanthus corcovadensis in mice. *Planta Med*. **61**, 329-324.

Senatore F, Mscisz A, Mrugasiewiez K, Gorecki P. (1989) Steroidal constituents and anti-inflammatory activity of the horse chestnut (Aesculus hippocastanum L.) bark. *Boll Soc Ital Biol Sper*. **65**, 137-142.

Suchalatha S, Devi CS. (2004) Effect of Arogh-a polyherbal formulation on the marker enzymes in isoproterenol induced myocardial injury. *Indian J. Clin. Biochem*. **19**, 184-189.

Suchalatha S, Thirugnanasambantham P, Maheswaran E, Devi CS. (2004) Role of Arogh- a polyherbal formulation to mitigate oxidative stress in experimental myocardial infarction. *Indian J. Exp. Biol*. **42**, 224-226.

Turner RA. (1971) Screening Methods in Pharmacology, *Academic Press*. 100-113.

Vendruscolo A, Takaki I, Bersani- Amado LE, Dantas JA, Bersani-Amado CA, Cuman RK. (2006) Anti inflammatory and antinociceptive activities of Zingiber officinale Roscoe essential oil in experimental animal models. *Ind. J. Pharmacol*. **38**, 58-59.

Vinegar R, Schreiber W, Hugo RJ. (1969) Biphasic development of carrageenan edema in rats. *J. Pharmacol. Exp. Ther*. **166**, 96-103.

Vogel HG. (2002) Drug discovery and evaluation, pharmacological Assay. *Springer*. **2**, 670.

Winter CA, Risley EA, Nuss GW. (1962) Carrageenin-induced edema in hind paw of the rat as assay for anti-inflammatory drugs. *Proceed of the soc for Exp Biol and Med*. **11**, 544-547.

Woolfe G, MacDonald AD. (1994) The evaluation of the analgesic action of pethidine hydrochloride. *J. Pharmacol. Exp. Ther.* **80**, 300.

Wheeler-Aceto H, Cowan A. (1991) Neurogenic and tissue mediated components of formalin induced edema agents actions. *Fitoterapia.* **34**, 264.