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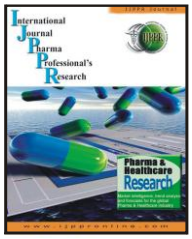

ANALGESIC AND ANTIINFLAMMATORY ACTIVITY OF A MARKETED POLY HERBAL FORMULATION (PHF)

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Abstract
The effect of PHF was investigated in experimental models of pain and inflammation. Analgesic activity of PHF (30,100,300, and 500 mg/kg, p.o.) was studied in mice using acetic acid induced writhing, tail immersion method and hot plate method. Anti-inflammatory activity of PHF (30, 100 and 300 mg/kg, p.o.) was studied in rats using carrageenan induced hind paw edema and formalin induced rat paw edema method. PHF (100, 300, and 500 mg/kg) significantly ($p<0.05$) reduced the number of writhing, increased latency to flick tail in tail immersion method and elevated the mean basal reaction time in hot plate method. PHF (30, 100, and 300mg/kg) significantly ($p<0.05$) inhibited carrageenan induced hind paw edema and formalin induced rat paw edema.

Keywords: PHF; Analgesic; Anti-inflammatory

Introduction
Since time immemorial, indigenous plants have been a major source of medicine. Prolonged administration of steroidal and nonsteroidal anti-inflammatory drugs are known to be associated with adverse effects. Herbal drugs have lesser side effects and are largely replacing the synthetic drugs. *Morus indica* [1], *Carpotroche brasiliensis*[2], *Drypetes molunduana*[3], *Araucaria bidwillii*[4], *Caesalpinia bonducella*[5], *Erythrina velutina*[6], *Comarum palustre*[7], *Mundula Sericea*[8], *Curcuma longa*[9] are some of the plants used for the relief of pain and inflammation. When tissue injury occurs, whether caused by bacteria, trauma, chemicals, heat, or any other phenomenon, mutiple substances that cause dramatic secondary changes in tissues are released by the injured tissues called as inflammation [10]. Arogh, a polyherbal ayurvedic formulation has been studied for its antioxidant property[11] and is also used in the treatment of myocardial infarction[12]. It is composed of nine-plant ingredients- *Nelumbo nucifera, Terminalia chebula, Zingiber officinale, Glycyrrhiza glabra, Hibiscus rosa-sinensis, Eclipta alba, Quercus infectoria, Hemidesmus indicus* and *Rosa damascene*[12] No study on its analgesic and anti-inflammatory activity has been reported. The present study is therefore an attempt to assess the PHF for its analgesic and anti-inflammatory activity using various animal models.

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Materials and Methods:
Chemicals and Drugs
Aspirin, Pentazocine, Carrageenan, Formalin were used in the study.

Test Samples and Standards
PHF (30, 100, 300, and 500 mg/kg), Carrageenan and Aspirin were prepared in 2% gum acacia suspension before oral administration. Formalin and Pentazocine were dissolved in water for injection before intraperitoneal administration.

Test Animals
Adult Swiss male albino mice (20-25 g) and albino rat(150-200gms) were procured from Institute of Animal Health and Veterinary Biological, Mhow, Indore, MP and used throughout the study. They were housed in microlon boxes in a controlled environment (temperature 25 ± 2^0C and 12 h dark/ light cycle) with standard laboratory diet and water *ad libitum*. The study was conducted after obtaining clearance from Institutional animal ethical committee.

Analgesic Activity

Acetic Acid Induced Writhing Method
In this method, mice in groups of six each were treated with vehicle, PHF (30,100,300, and 500 mg/kg, p.o.) and aspirin (20 mg/kg, p.o). Analgesic activity of PHF (30,100,300, and 500 mg/kg, p.o.) was assessed by counting the number of writhes induced by 0.6% acetic acid (10 ml/kg i.p.)[13,14]. Number of

writhes per animal was counted in the following 20 min.

Aspirin (20 mg/kg, p.o.) was used as a reference standard. Percentage protection against writhing was taken as an index of analgesia.

It is calculated as:

$$X_1 - X_2 / X_1 \times 1000$$

X_1= No of writhing in control group

X_2= No of writhing in treated group

Tail Immersion Method

Mice in groups of six each were treated with vehicle, pentazocine (17.5 mg/kg, i.p.) and

PHF (30, 100, 300, and 500 mg/kg, p.o.). The distal 2-3 cm portion of mouse-tail was immersed in hot water maintained at 55 ± 0.5 ºC[14]. 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 six 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 in hot plate maintained at a temperature of 55 ± 0.5 ºC[14]. The latency to lick the paw or jump from the hot plate was taken 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 30 sec.

Anti-inflammatory Activity

Carrageenan Induced Rat Paw Edema

The method of Winter *et al.*, (1962) was used to study acute inflammation. Five groups of six rats in each group were treated with vehicle, PHF (30, 100, and 300 mg/kg, p.o.), standard drug and combination of PHF (300 mg/kg) with Aspirin (20 mg/kg) one hour prior to Carrageenan injection. 0.1ml of 1% Carrageenan was injected into the subplantar tissue of left hind paw of each rat. Swelling of Carrageenan injected foot were measured at 0, 1, 2, 3, 4 h using Plethysmometer (UGO Basile, Italy) [15]. The right hind paw was injected with 0.1 ml of vehicle. Aspirin (20 mg/kg p.o.) was used as reference agent.

Formalin Induced Rat Paw Edema

Five groups of six rats in each group were treated with vehicle, PHF (30, 100, and 300 mg/kg, p.o.), standard drug and combination of PHF (300 mg/kg) with Aspirin (20 mg/kg) one hour prior to formalin injection. 0.05ml 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) [16.17], The right hind paw was injected with 0.1 ml of vehicle. Aspirin (20 mg/kg, p.o.) was used as reference agent.

Statistical Analysis:

All values are shown as mean ± SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnet's t test. $p<0.05$ was considered statistically significant.

Results and Discussion

Acetic Acid Induced Writhing Method

PHF (30, 100, 300, and 500 mg/kg, p.o.) significantly ($p<0.05$) reduced the number of writhing induced by acetic acid. Maximum percentage of inhibition of writhing response shown by PHF (500 mg/kg) was 49.35%, which was comparable to Aspirin (20 mg/kg). The observations are given in Table 1.

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

The readings are expressed as mean ± SEM. *$p<0.05$ as compared to control was considered significant. n=6

Tail Immersion Method

PHF (100, 300, and 500 mg/kg, p.o.) significantly ($p<0.05$) increased latency to flick tail in tail immersion method. The highest nociception inhibition of stimulus exhibited by PHF (500 mg/kg) was observed at 30 min. The observations are given in Table-2.

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)						
	0min	**15min**	**30min**	**45min**	**60min**	**90min**	**120min**
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	3.58±0.30	5.86±0.71*	7.03±0.60*	5.23±0.46*	4.91±0.34	4.18±0.29	3.90±0.26
PHF (300)	2.63±0.23	6.18±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	6.52±1.17*	7.53±1.51*	7.01±1.15*	5.62±1.08*	4.61±0.74*	3.57±0.73

*The readings are expressed as mean ± SEM. *p<0.05 as compared to control was considered significant. n=6*

Hot Plate Method

PHF (100, 300, and 500 mg/kg, p.o.) significantly ($p<0.05$) elevated the mean basal reaction time in hot plate method. The highest nociception inhibition of stimulus exhibited by PHF (500 mg/kg) was observed at 30 min. The observations are given in Table 3

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	6.81±0.61*	9.36±1.15*	5.41±0.36*	4.88±0.53*	3.90±0.39*	3.28±0.31
PHF (300)	2.57±0.20	7.82±0.46*	9.40±0.89*	5.16±0.34*	4.41±0.18*	3.78±0.26*	3.15±0.29
PHF (500)	2.38±0.18	9.17±0.62*	10.25±0.61*	8.14±0.52*	7.28±0.33*	5.91±0.22*	4.87±0.17*

*The readings are expressed as mean ± SEM. *p<0.05 as compared to control was considered significant. n=6*

Carrageenan Induced Rat Paw Edema

The PHF (30, 100, and 300 mg/kg, p.o.) significantly ($p<0.05$) inhibited carrageenan induced rat paw edema. Maximum inhibition of paw edema was observed in PHF (300 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.

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

*The readings are expressed as mean ± SEM. *p<0.05 as compared to control was considered significant. n=6*

Treatment (mg/kg)	Mean increase in paw volume (ml)					% Decrease in paw volume 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)	0.74±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*	1.24±0.04*	43.11
Aspirin (20)+PHF (300)	0.71±0.06	0.96±0.04*	1.06±0.06*	1.08±0.05*	1.09±0.05*	50.00

Formalin Induced Rat Paw Edema

The PHF (30, 100, and 300 mg/kg, p.o.) significantly ($p<0.05$) inhibited formalin induced rat paw edema. Maximum inhibition of paw edema was observed in PHF (300mg/kg) at 4 h when compared to the control group. Aspirin inhibited paw edema by 35.55%. The observations are given in Table 5.

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

Treatment (mg/kg)	Mean increase in paw volume (ml)					% Decrease in paw volume 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	0.66±0.02*	0.72±0.02*	0.80±0.02*	0.87±0.01*	35.55
PHF (30)	0.59±0.008	0.77±0.01*	0.85±0.006*	0.94±0.008*	1.01±0.007*	25.15
PHF (100)	0.52±0.02	0.75±0.03*	0.83±0.03*	0.92±0.01*	0.96±0.01*	28.89
PHF (300)	0.51±0.02	0.73±0.03*	0.82±0.02*	0.89±0.01*	0.93±0.03*	31.11
Aspirin(20) +PHF (300)	0.50±0.01	0.63±0.02*	0.70±0.02*	0.75±0.01*	0.79±0.02*	41.48

The readings are expressed as mean ± SEM. *$p<0.05$ as compared to control was considered significant. n=6

Discussion

The preliminary phytochemical screening of PHF showed the presence of alkaloids, saponins, steroids, flavonoids, carbohydrate, starch, and tannins[18]. 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 PGE2α in the peritoneal fluid[19.20]. 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. It is therefore likely that NSAIDS be more effective in inhibiting pain for such models. In our study, PHF (100, 300, and 500 mg/kg, p.o.) exhibited a significant analgesic effect in above models of pain.

Carrageenan induced rat paw edema has been a popular inflammatory model to investigate nonsteroidal anti-inflammatory effect of compounds[21]. It shows a biphasic effect[22]. 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 and 300 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[23], According to Yuh-Fung *et.al*.,[24], acute inflammation induced by formalin results from cell damage which provides the production of endogenous mediators. Edema produced by formalin was significantly ($p<0.05$) inhibited by PHF (30, 100 and 300 mg/kg, p.o.). Carrageenan was found to be more potent in inducing edema than formalin, indicating a more reliable model for inflammation.

Conclusion

It can be 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.

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