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Research Article

Evaluation of anti-inflammatory and analgesic activity of poly herbal formulation (PHF) in albino rats

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ABSTRACT

The effect of poly herbal formulation consisting of Gum acacia suspension of *Aegle marmelo* leaves*, Eugenia jambolana* seeds *and Bacopa monnieri* leaves (1:1:1) respectively was investigated for analgesic and anti – inflammatory activity in various experimental models of pain and inflammation. Analgesic activity of PHF (50, 100 mg/kg, I.P) was studied in rats using, tail immersion method and hot plate method. Anti-inflammatory activity of PHF (100, 150mg/kg, I.P) was studied in rats using Histamine induced edema and egg white induced rat paw edema method. PHF (100 mg/kg I.P) significantly ($p<0.01$) increased latency to flick tail in tail immersion method and elevated the mean basal reaction time in hot plate method compared to control (2ml of1%Gum acaia suspension). PHF (150mg/kg) significantly ($p<0.01$) inhibited histamine and egg white induced rat paw edema, compared to standard drug Indomethacin (20mg/kgI.P).

Keywords: PHF; Analgesic; Anti inflammatory; Indomethacin

INTRODUCTION

Inflammation is considered as a primary physiologic defense mechanism that helps body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli. An uncontrolled and persistent inflammation may act as an etiologic factor for many of these chronic illnesses (Kumar V *et al.*, 2004). Although it is a defense mechanism, the complex events and mediators involved the inflammatory reaction can induce, maintain or aggravate many diseases (Sosa S *et al.,* 2002). 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 (Ahamed *et al.,* 2005). 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 (Goodman and Gillman, 2001). A Polyherbal formulation contain three medicinal plants *Aegle marmelos leaves, Bacopa monnieri leaves* and *Eugenia jambolana Seeds* (1:1:1) was selected for our study. These constituents are used individually in folk medicine for the treatment of inflammation and pain. The analgesics and anti-inflammatory activity of these three plants has been mentioned in the literature (Channa S *et al.*, 2006; Chaudhuri N *et al.*, 1990; Ghangale G. R *e.tal*, 2008; Shankarnanth V *e.tal*, Viji V *et al.*, 2006;)No scientific study has been carried out so far on the combination. Hence, the present study was carried out to evaluate analgesic, anti-inflammatory activity of PHF in rats.

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MATERIALS AND METHODS

PLANTS MATERIAL

The plant material used in this study (*Aegle marmelos leaves, Eugenia jambolana seeds, Bacopa monnieri leaves)* PHF (1:1:1) was obtained as gift sample from Maddi laboratories, Secunderabad.

ANIMALS

Albino rats weighing around 100 - 200 g were procured from the National Institute of Nutrition, Hyderabad. The animals were kept in polypropylene cages (three in each cage) at an ambient temperature of 25 ± 20°C and 55 - 65% relative humidity 12 ± 1 h light and dark schedule was maintained in the animal house till the animals were acclimatized to the laboratory conditions and were fed with standard food pellets and water ad libitum. The animals were deprived of food 24h before experimentation and had free access to water. The animals were maintained under standard environmental conditions throughout the period of experimentation. The experiments were designed and conducted in accordance with the institutional guidelines. All procedures compiled with the norms of the animal ethics committee.

CHEMICALS USED

Histamine (Molychem, HYD), Indomethacin (Jagsonpal Pharmaceuticals Ltd.HYD), Gum acacia (Sd fine, HYD).

TEST SAMPLES AND STANDARDS

PHF (50, 100, 150, mg/kg, Indomethacin were prepared in 1% gum acacia suspension before oral administration. Histamine was dissolved in water for injection before intra-peritoneal administration.

ACUTE TOXICITY STUDY (Turner.R.A et al.**,** 1965)

Acute toxicity study was performed in accordance with OECD guidelines 425.No adverse effect or mortality was detected in albino rats up to 3 gm/kg, p.oof polyherbal 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.

EVALUATION OF ANALGESIC ACTIVITY

1. Tail flick method (D'Amour FE *et al.,* 1941)

Analgesic activity was also evaluated using Tail flick method. In this method, 1 to 2 cm of the tail of mice was immersed in warm water kept constant at 55°C. The reaction time was the time taken by the mice to deflect their tails. To evaluate the central analgesic effects of the polyherbal formulation, tail flick test was performed by time taken for rats to withdraw the tail when immersed in water maintained at 55±0.5° C was measured. The animals were divided into three groups of 6 animals each.

Group I - Control received 2ml of 1% Gum acacia suspension I.P

Group II - 1%Gum acaia suspension of PHF 50 mg/kg I.P.

Group III - 1%Gum acacia suspension of PHF 100 mg/kg body I.P. respectively

The animals are allowed to adapt to the cages for 30 min before testing. The lower 5 cm portion of the tail is marked. This part of the tail is immersed in a cup of freshly filled water of exactly 55 °C. Within a few seconds the rat reacts by withdrawing the tail. The reaction time is recorded in 0.5 s units by a stopwatch.- After each determination the tail is carefully dried. The reaction time is determined before and periodically after I.P administration of the, control test substance, and standard drug e.g., after 0.5, 1, 2, 3, 4 and 6 h. The cut off time of the immersion is 15 s. The withdrawal time of untreated animals is between 1 and 5.5 s. A withdrawal time of more than 6 s therefore is regarded as a positive response.

2. Eddy's hot plate method (Eddy NB *et al.*, 1953; Kulkarni SK *et al.*, 1999; Harborne JB, *et al.*, 1998.)

Analgesic activity of the polyherbal formulation was evaluated using analgesiometer. Analgesiometer employs Eddy's hot plate method., Eddy's hot-plate performs rapid and precise screening of analgesic drug properties on small-laboratory animals according to the hot-plate test. The alterations of animal pain sensitivity induced by a specific experimental context change and/or genetic manipulations can also be evaluated by using this method. In this method heat is used as a source of pain. Animals are individually placed on a hot plate maintained at constant temperature (55◦C) and the reaction of animals, such as paw licking or jump response is taken as the end point. Analgesics increase the reaction time. It evaluates thermal pain reflexes due to footpad contact with a heated surface. The animals were divided into three groups of 6 animals each.

Group I - Control received 2ml of 1% Gum acacia suspension I.P

Group II - 1%Gum acacia suspension of PHF (50 mg/kg I.P.)

Group III - 1%Gum acacia suspension of PHF 100 mg/kg body I.P. respectively

The animals were individually placed on the hot plate maintained at 55°C, one hour after their respective treatments. The response time was noted as the time at which animals reacted to the pain stimulus either by paw licking or jump response, whichever appeared first. The cut off time for the reaction was 15 seconds.

EVALUATION OF ANTI-INFLAMMATORY ACTIVITY (Parmar, N.S *et al.*, 1978; Nadkarni, K.M *et al.*, 1976).

Anti-inflammatory activity of the polyherbal formulation was assessed by using Plethysmograph. It is a glass tube of 20 mm internal diameter and one end fabricated to a glass tube with 0.5 mm bore. This tube is fused to a flexible tube and a pump (glass - syringe) and fixed to other end of the tube. This pump is used to adjust the level of mercury in both the flexible tube and graduated glass tube up to zero level.

1. Histamine induced paw oedema (Anonymous *et al.*, 1972; Purushothaman, *et al.*, 1987.)

FOR THE evaluation of anti inflammatory activity of PHF using Histamine induced paw model rats were selected and divided into four groups of 6 in each group. The paw oedema was produced by sub-plantar administration of 0.1 ml of a 0.1% freshly prepared solution of histamine into the right hind paw of rats of each group. The paw volume was recorded before (0 h) and 1 h after histamine injection. The animals were pretreated with

Group –I Control (2ml of 1%Gum acacia suspension I.P)

Group-II-- 1%Gum acacia suspension of PHF 100mg/kg I.P

Table 1: Effect of PHF on tail flick response in rats after immersion in 55° water baths

Treatment (mg/kg)	Basal reaction time (sec)				
	0min	30min	60min	90min	120min
Control(2ml of 1%Gum acacia suspension)	1.4±0.16	1.4±0.18	1 ± 0.23	1.1±0.19	0.9±0.20
PHF 50	2.2±0.61	2.5±0.22	2.7±0.22	2.4±0.01	2.9±0.82
PHF 100	3.5±0.56	3.8±0.64	3.9±0.85	4.1±0.69	4.2±0.71

Values are expressed as mean ± SEM. *p<0.01 as compared to control was considered significant. n=6

Table 2: Effect of PHF on rats using analgesiometer (hot plate method)

Treatment (mg/kg)	Basal reaction time (sec)				
	0min	15min	30min	60min	120min
Control(2ml of 1%Gum acacia suspension)	2±0.34	1.7±0.29	1.6±0.26	1.6±0.96	1.5±0.85
PHF 50	4±0.49	3± 0.89	4±0.19	4.5±0.45	4.5±0.92
PHF 100	4.5±0.45	4±0.41	4.1±0.91	4.5±0.25	5±0.25

Values are expressed as mean ± SEM. *p<0.01 as compared to control was considered significant. n=6

Group –III --. 1%Gum acacia suspension of PHF 150mg/kg I.P

Group-IV 1%Gum acacia suspension of Indomethacin 20mg/kg I.P

The drugs were administered intraperitoneally 1 h before eliciting paw oedema. The oedema was expressed as an increase in paw volume due to Histamine.

The percent inhibition of the inflammation is calculated using the formula and compared with control group.

$$\% \text{ Inhibition} = Vc - Vt / Vc \times 100$$

Vt and Vc edema volume in the drug treated and control groups respectively.

2. Fresh egg-white induced paw oedema (Arrigoni Martelli E *et al.*, 1977; Insel PA *et al.*, 1996.)

For the evaluation of anti inflammatory activity of PHF using Fresh –Egg white induced paw model rats were selected and divided into four groups of 6 in each group. The paw oedema was produced by sub plantar administration of 0.05 ml of undiluted fresh egg-white into the right hind paw of rats of each Group. The paw volume was recorded before (0 h) and 1 h after Fresh – Egg white injection. The animals were pretreated with the following

Group –I - Control (2ml of 1%Gum acacia suspension I.P)

Group-II - 1%Gum acacia suspension of PHF 100mg/kg I.P

Group –III -. 1%Gum acacia suspension of PHF 150mg/kg I.P

Group-IV - 1%Gum acacia suspension of Indomethacin 20mg/kg I.P

The drugs were administered intraperitoneally 1 h before eliciting paw oedema. The oedema was expressed as an increase in paw volume due to egg-white. The percent inhibition of the inflammation is calculated using the formula and compared with control group.

$$\% \text{ Inhibition} = \frac{Vc - Vt}{Vc} \times 100$$

Vt and Vc edema volume in the drug treated and control groups respectively.

STATISTICAL ANALYSIS

The results are expressed as means ± standard deviation (S.D.) and values were calculated for each group. A one way analysis of variance (ANOVA) followed by Dunnet's test for significance analysis using Graph Pad Prism software. The level of significance was found to be P<0.01.

RESULTS

Acute toxicity test

Intra-peritoneal administration of graded doses of the PHF (50, 100, 500, 1000 and 2000mg/kg I.P.) to rats did not produce any significant changes in behaviour, breathing, cutaneous effects, sensory nervous system responses or gastrointestinal effects during the observation period. No mortality was recorded in any group after 24 h of administering of the formulation. Mortality was observed with 1000mg/kg and 2000mg/kg after 48 hrs. So, we have selected the doses below 500mg/kg for our study.

ANALGESIC ACTIVITY

Tail flick method

In the tail flick test, latency to flick tail increased significantly (p<0.01) from 60 to 120 min after single peritoneal administration of *PHF,* and the highest nociception inhibition of stimulus exhibited by PHF (100 mg/kg) was observed at 120 min indicating dose and time dependant analgesic activity of the PHF compared to control group. The observations are given in Table-1.

Hot plate method

In the hot plate test, there was significant (p<0.01) increase in reaction time in *PHF* (50 &100mg/kg) from 0 to 60 min However, in the control group the duration

Table 3: Effect of PHF on inflammation in Histamine induced paw oedema

Treatment (mg/kg)	Mean increase in paw volume (ml)				% Decrease in paw volume at 60 min
	0min	15min	30min	60min	
Control(2ml of 1%Gum acacia suspension	0.52±0.10	0.21±0.40	0.34±0.10	0.25±0.10	-
PHF 100	0.32 ±0.24	0.25±0.25	0.25±0.20	0.15±0.14	40
PHF 150	0.24 ± 0.14	0.19±0.20	0.2±0.15	0.13±0.19	48
Indomethacin 20	0.21±0.33	0.2±0.24	0.22±0.381	0.10±0.14	60

Values are expressed as mean ± SEM. *p<0.01 as compared to control was considered significant. n=6

Table 4: Effect of PHF on inflammation in fresh egg-white induced oedema

Treatment (mg/kg)	Mean increase in paw volume (ml)				% Decrease in paw volume at 60 min
	0min	15min	30min	60min	
Control(2ml of 1%Gum acacia suspension	0.49±0.14	0.42±0.10	0.41±0.21	0.31±0.21	-
PHF 100	0.38±0.245	0.36±0.150	0.30±0.20	0.19±0.14	39
PHF 150	0.30±0.215	0.1±0.234	0.31±0.15	0.15±0.13	52
Indomethacin 20	0.24±0.238	0.25±0.149	0.14±0.21	0.14±0.12	55

Values are expressed as mean ± SEM. *p<0.01 as compared to control was considered significant. n=6

of reaction time did not increase significantly up to the end of the study period. Reaction time at 120 min of observation *PHF* [50mg/kg and 100mg/kg] as treated groups was significantly (p<0.01) higher compared to the control group indicating dose and time dependant anti nociceptive activity of the PHF. The highest nociception inhibition of stimulus exhibited by PHF (100 mg/kg) was observed at 120min compared to control group. The observations are given in Table 2

ANTI-INFLAMMATORY ACTIVITY

Histamine induced rat paw oedema method

The effect of the PHF (100mg/kg) and the reference drug on histamine-induced paw oedema was most pronounced 1h after histamine injection and the percentage inhibition was found to be 40%, while with the 150mg/kg dose of PHF showed highest activity at 1h after histamine administration with an inhibition of 48%. The anti-histaminic activity of the PHF increased with increase in the dose of the PHF. With 100mg/kg the significant reduction is not similar to the standard drug but when the dose is increased to 150mg/kg the reduction was significantly reduced (p<0.01) and was found to nearter to standard (indomethacin).The observations are given in Table 3.

Fresh egg white induced rat paw oedema

The effect of the PHF (100mg/kg) and the reference drug on **Fresh egg white induced rat paw oedema** was most pronounced 1h after histamine injection and the percentage inhibition was found to be 39%, while with the 150mg/kg dose of PHF showed highest activity at 1h after histamine administration with an inhibition of 52%. The anti-histaminic activity of the PHF increased with increase in the dose of the PHF. With 100mg/kg the significant reduction is not similar to the standard drug but when the dose is increased to 150mg/kg the reduction was significantly reduced (p<0.01) and was found to neater to standard (indomethacin).The observations are given in Table 4.

DISCUSSION

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. Analgesia is produced by liberating endogenous substances and many others that excite pain at nerve endings [Rao C.V et *al.*, 2005; Witkin L.B *et al.*, 1961]. The Poly-herbal formulation possesses central analgesic activity by increasing the latency to flick tail in tail immersion method and elevated the mean basal reaction time in hot plate method. It was found that the intensity of the analgesic effect was dose dependant manner. The inflammatory response is a physiological characteristic of vascular tissue. Increased permeability seen in the inflammatory reaction leads to exudation of fluid rich in plasma proteins, coagulation factors and injured tissues with subsequent edema at the site (Rang, H.P, 1965.). Exudation which is a consequence of vascular permeability is considered as major features of acute inflammation (Thirupathy K.P *et al.*, 2001**)**. Histamine and other mediators of inflammation increase vascular permeability at various times after injury. Chemically induced vascular permeability can causes an immediate reaction and its inhibitions suggests that the PHF administration may effectively suppress the oxidative phase of acute inflammation induced by undiluted fresh egg

white and histamine induced oedema.. The results also shows the effect of formulation on edematous response to histamine induced oedema AND egg white induced paw edema, provoking an inhibitory effect almost equal to that of standard Indomethacin Hence, it is speculated that apart from inhibition of chemical mediators of inflammation, *PHF may* also modulate the pain response in the central nervous system

As mentioned earlier, *PHF* contains 3 different constituents. A survey on the activities of the constituents revealed these constituents are used individually in folk medicine for the treatment of inflammation and pain. The analgesics and anti-inflammatory activity of these three plants has been mentioned in the literature (Channa S, *et al.,* 2006; Chaudhuri N *et al.*, 1990; Ghangale G. R *et al.,* 2008;Shankarnanth V *e.tal 2002 ;* Viji V *et al.,* 2006;)To conclude, the poly-herbal formulation possesses good analgesic, anti-inflammatory effects compared to individual constituents.

CONCLUSION

The results of the present study shows that the Poly herbal formulation possesses significant anti-inflammatory and analgesic activities in all the tested various inflammatory experimental animal models such as Histamine induced oedema and Fresh egg white induced oedema indicating inhibition of all phases of inflammation. The Poly-herbal formulation possesses central analgesic activity which was evaluated using hot plate method and Tail flick method. Further there is a scope to determine the active constituents of PHF and to determine its mechanism of action.

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