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Evaluation of Anti-Inflammatory Activity of Polyherbal Formulation

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ABSTRACT

Objectives: The present investigation was carried out to evaluate the anti-inflammatory activity of polyherbal formulation (PHF) of aqueous extracts of *Mimusopselengi*, *Strobilanthesbarbatus*, *Indigoferazollingeriana*, *Dilleniaindica*Materials and methods: Swiss albino mice of either sex and Wistar strain of albino rats of either sex were used for the study. In vitro anti-inflammatory activity of PHF was studied by Inhibition of albumin denaturation, Anti-proteinase action, Membrane Stabilization Action, Heat induced haemolysis, Hypotonicity-induced haemolysis, Anti-lipoxygenase activity. Carrageenan induced Paw edema and Hot Plate Tests were used for in vivo study. Aspirin (100ug/ml), Diclofenac (100ug/ml), Indomethacin (100ug/ml) were used as standards.Results: PHF at 250, 500, and 1000mg/kg showed significant ($p<0.05$) inhibition of protein denaturation. A significant ($p<0.05$) inhibition on anti-proteinase action was observed in a dose dependent manner. PHF exhibited significant ($p<0.05$) membrane stabilizing action in a dose dependent manner. PHF offered significant protection ($p<0.05$) for the erythrocyte membrane against lysis, induced by heat in a dose dependent manner. PHF offered protection significantly for the erythrocyte membrane against lysis induced by hypotonic solution. PHF exhibited significant ($p<0.05$) anti- lipoxygenase inhibition. Edema suppressant effect of PHF treated group was found to be significant ($p<0.05$) on both phases of inflammation when compared to control. Conclusion: The study revealed that PHF has promising features for suppressing inflammation.

INTRODUCTION

Inflammation is an important pathologic and complex process. It is a defensive response to injury. The warmth, redness, swelling, and pain are the cardinal signs of "inflammation" [1]. It is caused by a series of cellular and tissue responses to some injurious agent. Agents that provoke inflammation are either physical agents, chemical agents or biological agents. The sequence of events following such injury involve blood vessel dilation, increase in vascular permeability, transudation margination of leucocytes, diapedesis, emigration, exudation and destruction of agent. Blood cellular components like neutrophils, monocytes, lymphocytes, vasoactive amines, kinins, factor XII, complement cascade (c1-c9), prostaglandins mediate or influence a number of responses associated with inflammation. There are two fundamental types of inflammation: acute and chronic. Acute inflammation is characterized by a rapid onset, short duration, and profound signs and symptoms. On the other hand, chronic inflammation is characterized by a slow onset, long duration, and less obvious signs and symptoms. In addition to the two basic forms (acute and chronic), there are two others that appear less commonly: subacute and granulomatous chronic inflammation [2] Subacute inflammation is a well defined form that has some clinical features of acute and some of chronic inflammation. Granulomatous chronic inflammation, as its name signifies, is a special form of chronic inflammation, associated with tuberculosis. Many herbs and herbal medicines have been used to cure many disorders including inflammatory diseases. An effort has been made here to investigate the anti-inflammatory activity of polyherbal formulation. The PHF was formulated using the herbs which have known anti-inflammatory effects. Aqueous extracts of *Mimusopselengi*, *Strobilanthesbarbatus*, *Indigoferazollingeriana*, *Dilleniaindica* were used in the preparation of PHF.

MATERIALS AND METHODS

Collection of plant material and preparation of extracts:

The test plants selected for this study, *Mimusopselengi*L., *Strobilanthesbarbatus*Nees, *Indigoferazollingeriana*Miquel, *Dilleniaindica*L were collected from ABS botanical gardens, Karipatti and authenticated by Dr. D. Arulbalachandran, Asst. Professor, Dept. of Botany, School of Life Sciences, Periyar University, Salem, Tamilnadu

Preparation of Polyherbal extract:

Decoction of Herbal powder:

Each plant powder (125g) was mixed thoroughly to make a polyherbal powder of 500g. Polyherbal powder (500g) was mixed with 4000 ml of sterile water and left as such for 30 min. Later, mixture was boiled until total volume becomesone fourth of its actual volume (i.e. 1000 ml). The mixture was cooled and strained. Filtrate will be incorporated to make polyherbal extract. During administration,composition was designed as decoction with vehicle (water) in 1:5 ratios. Clarity of the solution was properly observed visually for solubility. During administration, PHF was shaken well and used.

Phytochemical screening of the PHF:

PHF was subjected to Preliminary phytochemical screening for the presence of various bioactive components. The results provided the evidence for the presence of Carbohydrates, Proteins & Amino acids, Alkaloids, Glycosides, phenolics /Tannins, Flavonoids, Saponins, Fixed oils/Fats, Steroids. Results are tabulated [Table 1].

Animals:

Swiss albino mice (weighing 25-30 g) of either sex, 6-7 weeks of age were used in hot plate and writhing tests. The animals were housed under standard laboratory conditions with food and water provided ad libitum. Six mice were used in each experiment. Wistar strain of albino rats (200-250 g) of either sex were used for this carrageenan experiment. The animals were housed in standard metal cages and provided with food and water ad libitum. Six animals were used in each experiment.

Glassware and Chemicals:

All the glassware were cleaned, dried and stored in hot-air oven at 80ºC and used for the experiment throughout the study period. Reagent quality chemicals and glass distilled water were used in this study, wherever necessary.

Acute Toxicity Studies

The acute oral toxicity study [4] was carried out as per the guideline set by the Organization for Economic Co-operation and Development (OECD guidelines 425) received from the

Committee for the Purpose of Control and Supervision of Experiments on Animals (516/01/A/CPCSEA.)

Animals:

Swiss albino mice (weighing 25-30 g) of either sex, 6-7 weeks of age were used in hot plate and writhing tests. The animals were housed under standard laboratory conditions with food and water provided ad libitum. Six mice were used in each experiment. Wistar strain of albino rats (200-250 g) of either sex were used for this carrageenan experiment. The animals were housed in standard metal cages and provided with food and water ad libitum. Six animals were used in each experiment.

Assessment of Anti Inflammatory Activity:

Inhibition of albumin denaturation: The anti-inflammatory activity of Polyherbal formulation was studied by using inhibition of albumin denaturation technique which was studied according to Mizushima et al and Sakat et al followed with minor modifications. The reaction mixture consists of differnt concentrations of PHF and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount of 1N HCl. The samples were incubated at 37ºC for 20 min and then heated to 51ºC for 20 min, after cooling the samples the turbidity was measured at 660 nm (UV Visible Spectrophotometer Model 371, Elico India Ltd). The experiment was performed in triplicate [5]. The Percentage inhibition of protein denaturation was calculated as follows:

$$\text{PercentageInhibition} = \frac{\text{Absorbance control} - \text{Absorbance sample}}{\text{Absorbance control}} \times 100$$

Anti-proteinase action

The test was performed according to the modified method of Oyedepo et al [12] and Sakat et al. The reaction mixture (2 ml) was containing 0.06 mg trypsin, 1 ml 20 mMTrisHCl buffer (pH 7.4) and 1 ml test sample of different concentrations (100 - 500 µg/ml). The mixture was incubated at 37^{o}C for 5 min and then 1 ml of 0.8% (w/v) casein was added. The mixture was incubated for an additional 20 min. 2 ml of 70% perchloric acid was added to arrest the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm against buffer as blank. The experiment was performed in triplicate. The percentage inhibition of proteinase inhibitory activity was calculated [6].

$$\text{Percentage Inhibition} = \frac{\text{Absorbance control} - \text{Absorbance sample}}{\text{Absorbance control}} \times 100$$

Membrane Stabilization Action:

Blood was collected from healthy human volunteers who had not taken any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of sterilized Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% sodium chloride). The blood was centrifuged at 3000 rpm and packed cells were washed with isosaline (0.9% w/v NaCl) and a 10% suspension was made with isosaline. Various concentrations of the PHF were prepared (250, 500 and 1000 µg/ml) using distilled water and to each concentration 1 ml phosphate buffer, 2 ml hyposaline, and 0.5 ml HRBC suspension were added. These were incubated at 37° for 30 min and centrifuged at 3000 rpm for 20 min. The hemoglobin content in the supernatant solution was estimated spectrophotometrically at 560 nm. Indomethacin (100 µg/ml) was used as the reference standard and a control was prepared omitting the extracts. The percentage hemolysis was calculated by assuming the hemolysis produced by the control group as 100% [7].

The percentage of HRBC membrane stabilization or protection was calculated using the formula,

$$\text{Percentage Protection} = \frac{\text{OD Observed} - \text{OD OF Treated sample}}{\text{OD Of control}} \times 100$$

Heat inducedhaemolysis

The reaction mixture (2 ml) consisted of 1 ml test sample of different concentrations (250-1000µg/ml) and 1 ml of 10% RBCs suspension, instead of test sample only saline was added to the control test tube. Aspirin was used as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56ºC for 30 min. At the end of the incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicates for all the test samples.[8]

The Percentage inhibition of Haemolysis was calculated as follows:

$$\text{Percentage Inhibition} = \frac{\text{Absorbance control} - \text{Absorbance sample}}{\text{Absorbance control}} \times 100$$

Hypotonicity-induced haemolysis

Different concentration of extract (250-1000µg/ml),reference sample, and control were separately mixed with 1ml of phosphate buffer, 2 ml of hyposaline and 0.5 ml of HRBC suspension. Diclofenac sodium (100µg/ml) was used as a standard drug. All the assay mixtures were incubated at 37^{o}C for 30 minutes and centrifuged at 3000 rpm. The supernatant liquid was decanted and the haemoglobin content was estimated by a spectrophotometer at 560 nm. The percentage hemolysis was estimated by assuming the haemolysis produced in the control as 100% [9].

$$\text{Percentage Protection} = \frac{\text{OD Observed} - \text{OD OF Treated sample}}{\text{OD Of control}} \times 100$$

Anti-lipoxygenase activity

Anti-Lipoxygenase activity was studied using linoleic acid as substrate and lipoxidase as enzyme. Test samples were dissolved in 0.25 ml of 2M borate buffer pH 9.0 and added 0.25 ml of lipoxidase enzyme solution (20,000 U/ml) and incubated for 5 min at 250C. After which, 1.0ml of lenoleic acid solution (0.6mM) was added, mixed well and absorbance was measured at 234 nm. Indomethacin was used as reference standard [10].

The percent inhibition was calculated from the following equation,

$$\% \text{ inhibition} = \frac{\text{Absorbance control} - \text{Absorbance sample}}{\text{Absorbance control}} \times 100$$

A dose response curve was plotted to determine the IC_{50} values. IC_{50} is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity. All tests and analyses were run in triplicate and averaged

***In vivo* anti-inflammatory activity:**

Carrageenan induced Paw Edema:

Carrageenan induced rat paw edema model was used to study the *In vivo* anti-inflammatory activity of extracts. The extracts were suspended in distilled water using 1% SCMC as suspending agent. Male Wistar rats were divided into six groups each composed of six animals. Group I: Control animals received (1% SCMC, 10 ml/kg, p.o.) Group II: Animals received extract at the dose of 250 mg/kg p.o. Group III: Animals received extract at the dose

of 500mg/kg p.o. Group IV: Animals received extract at the dose of 1000mg/kg p.o. Group V: Animals received standard indomethacin (10 mg/kg, p.o.). Paw edema was induced by injecting 0.1 ml of 1% carrageenan in physiological saline into sub plantar tissues of hind paw of each rat. The extract at the dose of 200 and 400 mg/kg were administered orally 30 min prior to carrageenan administration. The paw volume was measured at intervals of 60, 120, 180, and 240 min by the mercury displacement method using a plethysmograph. Percent inhibition (%IE) of edema was calculated using the equation [11],

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

Where *V*c is the inflammatory increase in paw volume in control group of animals and *V* is the inflammatory increase in paw volume in drug treated animals. Inhibition of paw volume in drug treated group was compared with the carrageenan control group, whereas indomethacin (10 mg/kg p.o.) was used as reference drug.

Hot Plate Test:

The hot-plate test (Hot/Cold Plate Model-35100-001, UGO Basile, Italy) was employed for measurement of analgesic activity as previously described by Lanhers et al. and modified by Ojewole. The temperature was regulated at $55 \pm 1°C$. Mice of either sex were divided into four groups consisting of six animals in each group. The mice of each group were placed in the beaker (on the hot plate) in order to obtain its response to electrical heat induced pain stimulus. Licking of the paws or jumping out of the beaker was taken as an indicator of the animal's response to heat-induced pain stimulus. The time for each mouse to lick its paws or jump out of the beaker was taken as reaction time (in second). Before treatment, the reaction time was taken once. The mean of this determination constituted initial reaction time before treatment of each group of mice. Each of the test mice was thereafter treated with either distilled water (DW), Diclofenac sodium (10 mg/kg BW) or PHF at the doses of 250 ,500 and 1000 mg/kg BW orally. Thirty minutes after treatment, the reaction times of each group of mice were again evaluated five times individually on one hour interval [12]. Percent analgesic score was calculated as,

$$\text{Percent Analgesic score} = \frac{Ta - Tb}{Ta} x\ 100$$

Where, T_b = Reaction time (in second) before drug administration; T_a = Reaction time (in second) after drug administration.

Statistical analysis:

The statistical analysis was performed using Graph Pad prism version 5 and 7.4. The statistical significance of differences among various experimental groups was calculated by one-way ANOVA. All the values are expressed as mean ± S.D. Results were considered statistically significant when $p<0.05$

RESULTS AND DISCUSSION

Phytochemical screening: Implications of our study revealed the presence of carbohydrates, proteins and amino acids, alkaloids, glycosides, phenols, flavonoids and absence of fixed oils and steroids

Table 1: Phytochemical Screening of PHF*:*

Name of the Test	
Carbohydrates	
Molisch's Test	+
Bial's Test	+
Proteins & Amino acids	
Ninhydrin Test	+
Xanthoprotein Test	+
Millon's Test	+
Alkaloids	
Mayer's Test	+
Dragendroff's Test	+
Glycosides	
Borntrager's Test	+
Phenolics -	
Ferric Chloride Test	+
Flavonoids	
Alkaline reagent Test	+
NH_4OH Test	+
Fixed oils/Fats	
Spot Test	-
Steroids	-
Libermann-Burchard Test	-

Inhibition of albumin denaturation:

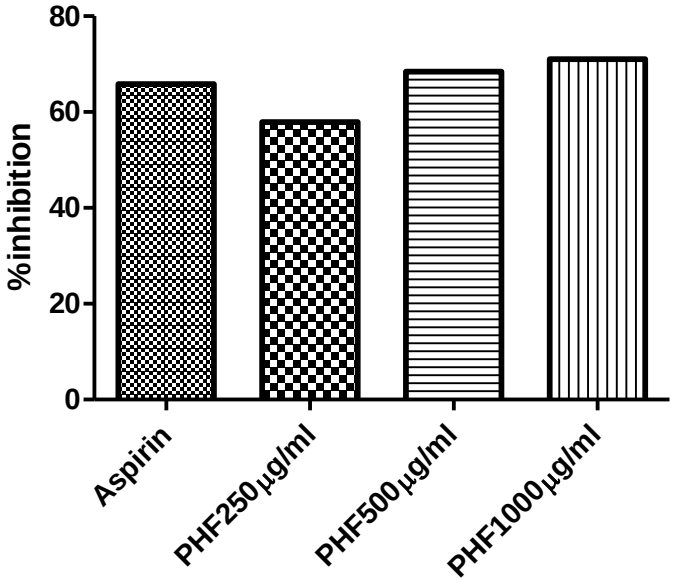
Protein Denaturation is a process in which proteins lose their complex structure by application of external stress, there by lose their biological function when denatured. Denaturation of proteins is a well documented cause of inflammation. PHF significantly ($p<0.05$) inhibited heat induced albumin denaturation. PHF at concentrations of 250, 500, and 1000µg/ml showed 57.9, 68.4, 71% inhibition of protein denaturation respectively. Aspirin, a standard anti- inflammatory drug showed the maximum inhibition of 65.8% at the concentration of 100 µg/ml compared with control ($p<0.01$) [Table2].

Table 2: Inhibition of albumin denaturation:

Treatment	Absorbance at 660 nm	% inhibition of protein denaturation
Control	0.38±0.05	-
Aspirin	0.13±0.01**	65.8
PHF (250 µg/ml)	0.16±0.02*	57.9
PHF (500 µg/ml)	0.12±0.07*	68.4
PHF (1000 µg/ml)	0.11±0.07*	71

Values expressed in mean ±SD,(n=6) Significant *P<0.05; **P<0.01 compared to control

Fig:1Percentage inhibition on Albumin denaturation



Proteinase Inhibitory Action:

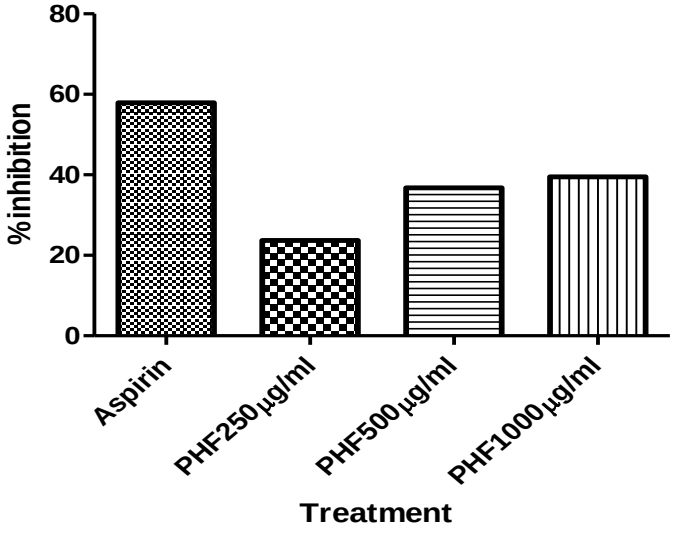
Neutrophils which are localized at lysosomes are known to be a rich source of serine proteinase. It was previously reported that leukocytes proteinase play an important role in the development of tissue damage during inflammatory reactions and significant level of protection was offered by proteinase inhibitors[16]. PHF at concentrations of 250, 500, 1000 µg/ml has shown 23.7, 36.8, 39.5% inhibition on anti-proteinase action significantly in a dose dependent manner. Aspirin, standard exhibited significant inhibition of 57.9 % compared with that of the control. Results were tabulated [Table 3].

Table 3: Inhibition of anti -proteinase action

Treatment	Absorbance 660 nm	% inhibition of anti–proteinase action
Control	0.38±0.09	-
Aspirin	0.16±0.01**	57.9
PHF (250 µg/ml)	0.29±0.01*	23.7
PHF (500 µg/ml)	0.24±0.03*	36.8
PHF (1000 µg/ml)	0.23±0.02*	39.5

Values expressed in mean ±SD (n=6), Significant *P<0.05; **P<0.01 compared to control

Fig 1.2 Percentage inhibition on Anti-proteinase action



Membrane stabilization:

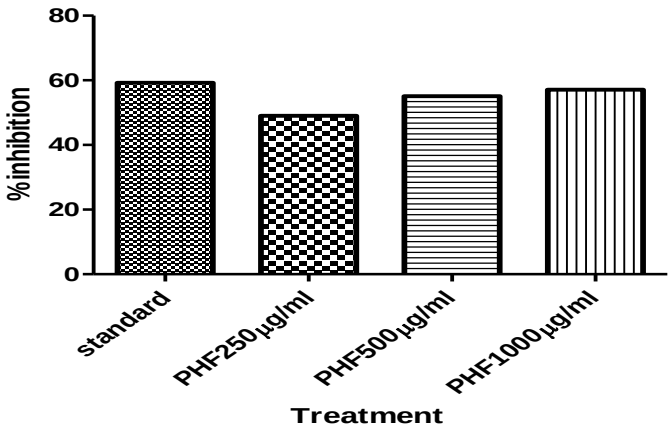
The HRBC membrane stabilization has been used as a method to study the *in vitro*anti inflammatory activity because the erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may well stabilize lysosomal membranes. Stabilization of lysosomal is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil, such as bacterial enzymes and proteases, which causes further tissue inflammation and damage upon extracellular release. The lysosomal enzymes released during inflammation produce various disorders. The extracellular activities of these enzymes are said to be related to acute or chronic inflammation. The nonsteroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane [19]. PHF exhibited significant membrane stabilizing action in a dose dependent manner. PHF at 1000 mg/kg offered 57.1% of protection and the standard offered 59.2% of protection

Table 4: Membrane Stabilization:

Treatment	Absorbance at 560 nm	% inhibition
Control	0.49±0.02	
Standard	0.20±0.001**	59.2
PHF (250 mg/kg)	0.25±0.001*	49
PHF (500 mg/kg)	0.22±0.001*	55.1
PHF (1000 mg/kg)	0.21±0.001*	57.1

Values expressed in mean ±SD, (n=6) Significant *P<0.05; **P<0.01 compared to control

Fig1.3Effect of PHF On membrane stabilization



Heat Induced Haemolysis:

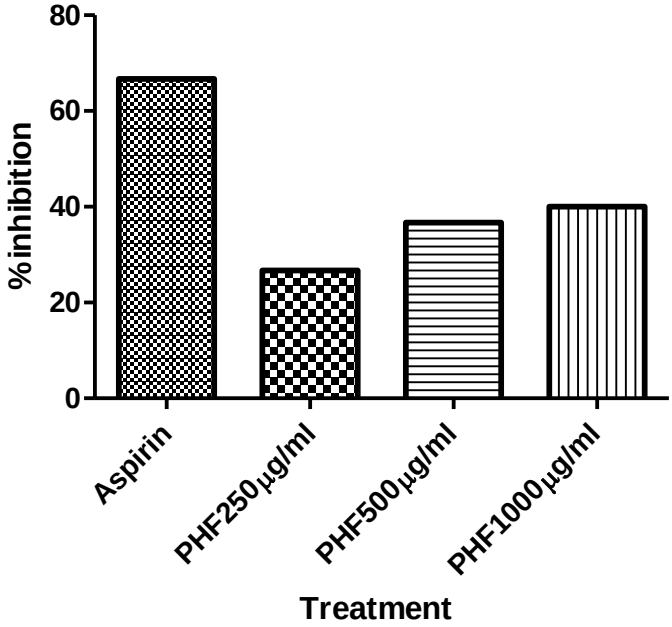
The extract was effective in inhibiting the heat inducedhaemolysis at different concentrations. The results showed that PHF at concentration 250, 500, 1000 µg/ml protect significantly ($p<0.05$) the erythrocyte membrane against lysis induced by heat (Table 5). PHF showed Dose dependent effect on erythrocyte membrane. Aspirin 100 µg/ml offered a significant ($p<0.01$) protection against damaging effect of heat solution.

Table 5: Heat inducedhaemolysis:

Treatment	Absorbance at 660 nm	% inhibition of haemolysis
Control	0.30±0.03	-
Aspirin	0.10±0.06 **	66.7
PHF (250 µg/ml)	0.22±0.07 *	26.7
PHF (500 µg/ml)	0.19±0.03*	36.7
PHF (1000 µg/ml)	0.18±0.03*	40

Values expressed in mean ±SD,(n=6) Significant *P<0.05; **P<0.01 compared to control

Fig 1.4 Percentage inhibition of Heat induced haemolysis by PHF



Hypotonicity Induced Haemolysis:

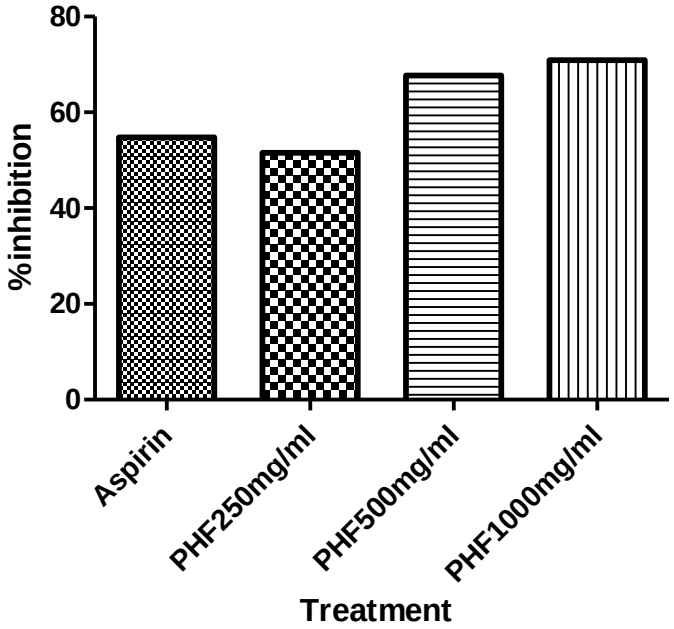
The results showed that PHF at 200, 500 and 1000µg/ml exhibited 51.6, 67.7, 70.9% inhibition of haemolysis respectively. PHF offered protection significantly the erythrocyte membrane against lysis induced by hypotonic solution (Table 6). Diclofenac sodium (100 µg/ml) exhibited 54.8% inhibition. Protection offered by PHF is in a dose dependent manner and is higher than the standard.

Table 6: Hypotonicity induced haemolysis:

Treatment	Absorbance at 660 nm	% Inhibition of haemolysis
Control	0.31±0.02**	-
Diclofenac Sodium	0.14±0.01**	54.8
PHF (250 µg/ml)	0.15±0.09*	51.6
PHF (500 µg/ml)	0.10±0.02*	67.7
PHF (1000 µg/ml)	0.09±0.02*	70.9

Values expressed in mean ±SD, (n=6) Significant *P<0.05; **P<0.01 compared to control

Fig 1.5 Percentage inhibition of Hypotonicity induced haemolysis by PHF



Anti-lipoxygenase activity:

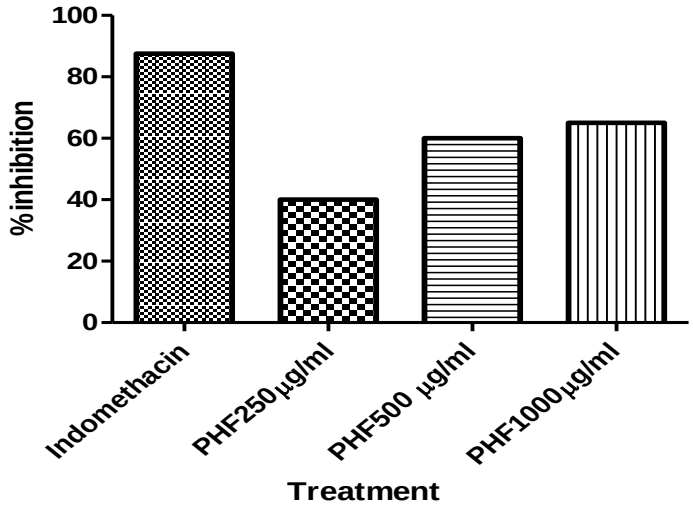
The establishment of new *in vitro* test systems has stimulated the screening of plants aiming to find leads for the development of new drugs. The plant lipoxygenase pathway is in many respects equivalent to the 'arachidonic acid cascades' in animals [20]. For this reason, the in vitro inhibition of lipoxygenase constitutes a good model for the screening of plants with anti-inflammatory potential [21]. LOXs are sensitive to antioxidants and the most of their action may consist in inhibition of lipid hydroperoxide formation due to scavenging of lipidoxy or lipid peroxy- radical formed in course of enzyme peroxidation. This can limit the availability of lipid hydroperoxide substrate necessary for the catalytic cycle of LOX. PHF at 250, 500 and 1000 µg/ml, exhibited 40, 60, and 65% of anti-lipoxygenase inhibition respectively. PHF exhibited dose dependent percentage inhibition of lipoxygenase activity. From these results, the strongest inhibition was obtained at concentration 1000µg/ml. The standard Indomethacin showed an inhibition of 87.5% at a concentration of 100µg/ml.

Table 7: Anti-Lipoxygenase activity:

Treatment	Absorbance at 660 nm	% inhibition of lipoxygenase activity
Control	0.40±0.02	-
Indomethacin	0.05±0.05**	87.5**
PHF (250 µg/ml)	0.24±0.05*	40
PHF (500 µg/ml)	0.16±0.02*	60
PHF (1000 µg/ml)	0.14±0.02*	65

Values expressed in mean ±SD,(n=6) Significant *P<0.05; **P<0.01 compared to control

Fig 1.6Percentage inhibition of Anti-lipoxynase activity by PHF



***In vivo*anti inflammatory activity:**

Carrageenan induced rat paw edema:

The anti-inflammatory effect of the formulation on carrageenan induced rat paw edema is presented in table 8.The development of carrageenan induced edema is bi-phasic, the 1st phase is mediated by the release of histamine and serotonin with a peak value at 1h: and the second phase is related to the release of prostaglandins with a peak value at 3h. Edema suppressant effect of PHF 250 mg/kg, 500 mg/kg, 1000 mg/kg treated group was found to be significant on both phases of inflammation when compared to control. The 250 mg/kg 500 mg/kg, and 1000 mg/kg doses showed maximum anti-edematous effect of about 58.4%, 67.5%, and 68.8% respectively at 4h after carrageenan administration.

Table 8: Carragenan induced Paw edema:

Group	60 min	120 min	180 min	240 min
Control	0.35±0.002	0.54±0.001	0.72±0.001	0.77±0.002**
Standard	0.21±0.001**	0.31±0.002**	0.26±0.003**	0.18±0.001**
PHF(250mg/kg)	0.26±0.001*	0.37±0.002**	0.37±0.003*	0.32±0.002*
PHF(500mg/kg)	0.23±0.003*	0.35±0.002*	0.33±0.003*	0.25±0.001*
PHF(1000mg/kg)	0.22±0.003*	0.34±0.002*	0.32±0.003*	0.24±0.001*

Values expressed in mean ±SD,(n=6) Significant *P<0.05; **P<0.01 compared to control

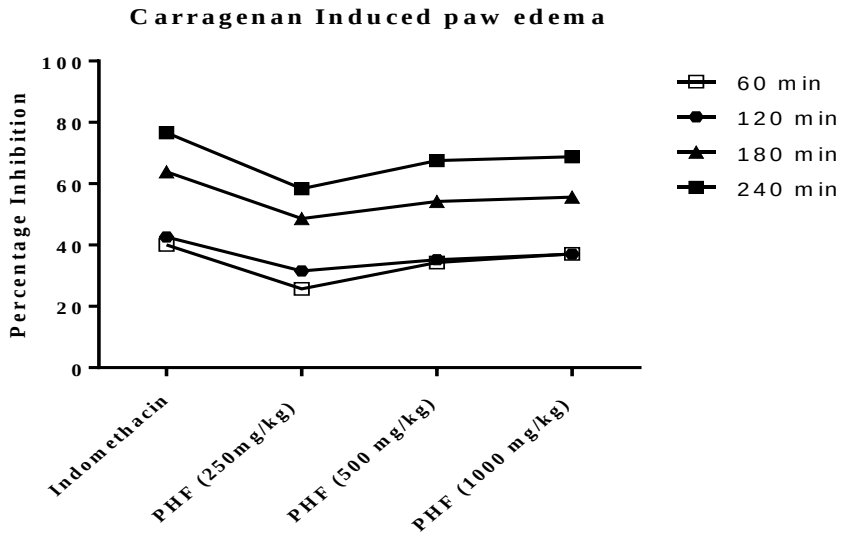


Fig 1.7: Effect of PHF on Carrageenan induced paw edema

Hotplate test

The anti-inflammatory activity was performed by Hotplate method induced writhing test which shows dose significant ($p<0.05$) inhibition. The percent pain inhibition seen by the PHF resulted to be significant within 240 minutes of pain latency.

Table 9: Hot plate Test

Group	0min	30min	60min	120min	180min	240min
	% inhibition					
Control	10.7±0.84	9.66±0.93	8±0.81	6.62±0.64	5.52±0.5	5±0.44
Standard	9.22±0.52**	11.12±1.00**	12.45±0.94**	14.2±1.07**	15.86±0.67**	12.52±0.69 **
PHF (250 mg/kg)	8.23±0.34*	9.24±0.42*	0.62±0.37*	12.24±0.15*	13.58±0.22*	11.26±0.02*
PHF (500 mg/kg	7.65±0.6 *	8.72±0.48*	10.35±0.43*	12.14±0.20 *	13.32±0.14*	10.12±0.24*
PHF(1000mg/kg)	7.32±0.61*	8.11±0.49*	10.33±0.42*	12.12±0.24 *	13.21±0.12*	10.04±0.23 *

Values expressed in mean ±SD, (n=6) Significant *P<0.05; **P<0.01 compared to control

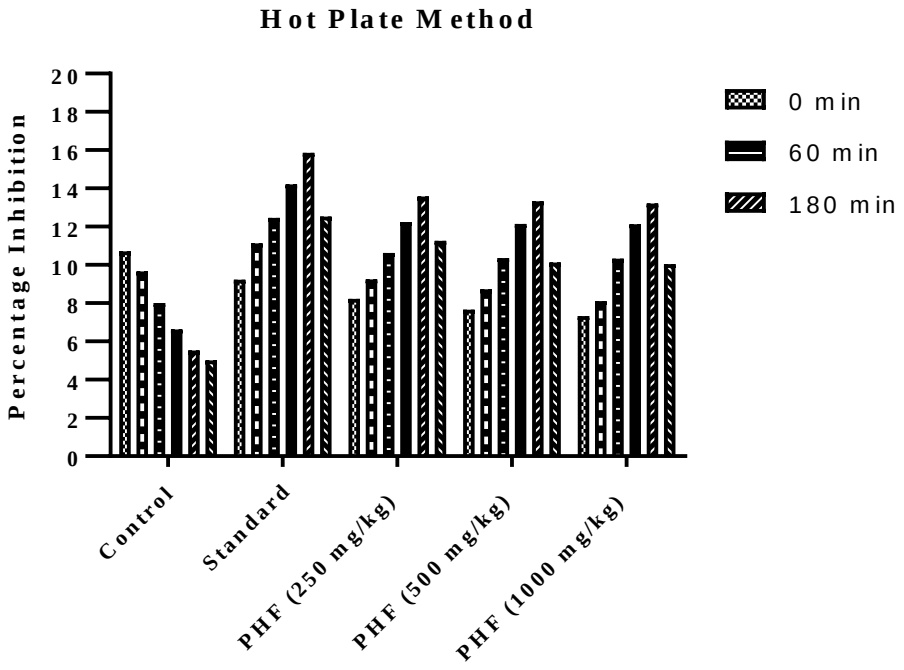


Fig 1.8: Effect of PHF on Hotplate test

CONCLUSION

Our findings based on *in vitro* and *in vivo* study indicate that the PHF possess anti-inflammatory properties. The significant anti-inflammatory activity evinced by the PHF merits further investigation of PHF for inflammatory diseases in humans.

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