

Anti-arthritis and anti-inflammatory activity of a polyherbal formulation against Freund's complete adjuvant induced arthritis in Wistar rats

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Piper longum L. (Piperaceae), *Clerodendrum indicum* (L.) Kuntze (Lamiaceae), *Acorus calamus* L. (Acoraceae) are traditionally used for the treatment of rheumatism, arthritis and other inflammatory conditions in the traditional medicine of India. The present study aimed to investigate the anti-arthritis effect of a polyherbal formulation (PHF) and its underlying mechanism in adjuvant induced arthritis (AIA). Arthritis was induced by intradermal injection of complete Freund's adjuvant (0.1 ml) into the left hind paw of the Wistar albino rats. PHF (100 & 200 mg/kg b.wt) and prednisolone (PDL) (5 mg/kg b.wt) were administered orally from 1st day to 28th day after adjuvant induction. Induction of arthritis significantly increased hind paw volume (HPV), levels of reactive oxygen species (LPO and NO), and inflammatory cytokines (TNF- α , IL-1 β and IL-6) with subsequent decrease in the anti-oxidant status (GSH, SOD and CAT) in arthritic rats compared to controls. Furthermore, the mRNA expression of inflammatory enzymes (iNOS and COX-2), and transcription factor (NF- κ B) was found upregulated in the joint tissues of arthritic rats in RT-PCR analysis. On the other hand, the above said imbalances were reverted back effectively to near normal following supplementation with PHF which was supported by the histopathological examination with decreased infiltration of inflammatory cells and synovial hyperplasia eventually protecting further damage of the affected joints. In conclusion, these findings showed that PHF exerted beneficial effects on rheumatoid arthritis in rats.

Keywords: Rheumatoid arthritis, Oxidative stress, Antioxidants, Inflammation, Cytokines.

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Rheumatoid arthritis (RA) is an autoimmune and chronic inflammatory disease of joints characterized by inflammatory cell infiltration, proliferation of synovial tissue, and bone destruction¹. Pain, stiffness, swelling, deformities, and loss of joint function are common manifestations in patients with RA¹. It is a debilitating condition occurring at any age, peaking between the ages of 35 and 50 years². Several lines of recent evidence have indicated that proinflammatory cytokines such as tumor necrosis factor- α (TNF- α), interleukin (IL)-1 β , IL-6, IL-17, IL-18, and IL-23 and inflammatory enzymes like inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) produced by the T cells and macrophages are reported to play a pivotal role in the pathogenesis of RA³. The pharmacological management of RA principally relies on treatment regimens that include the use of non-steroidal anti-inflammatory drugs (NSAIDs), disease-

modifying anti-rheumatic drugs (DMARDs), corticosteroids, and biologics. NSAIDs are directed towards the control of pain and swelling but have not been shown to inhibit the progression of the disease. Although these classes of medications are well tolerated for short periods, but their long term use is overshadowed by their untoward effects such as gastric perforation, cardiovascular complications, renal morbidity and gastrointestinal ulcers⁴. Corticosteroids are potent suppressors of the inflammatory response in many diseases; unfortunately, their dose dependent side effects are common that include thinning of the skin, cataracts, osteoporosis, hypertension and hyperlipidaemia⁵. The use of biologics has many limitations due to expensive costs, hypersensitivity, immunosuppression and infections. In addition, 30-50 % of patients on biologics do not have an expected clinical response⁷. Furthermore, due to chronic nature of disease, high cost and severe side effects associated with

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conventional treatment, 60-90 % of RA patients seek complementary and alternative medicine for symptomatic relief⁶. Numerous herbal formulations and their active ingredients are available in the market, with claims to afford protection against rheumatoid arthritis⁴. This growing interest in alternative medical practices clearly indicates a need for more safer and effective anti-RA botanicals from traditional medicine. *Piper longum* L. has been used as herbal remedy for several ailments. Traditional usage of the plant includes treatment of bronchitis, asthma and cold, as counter-irritant and analgesic. It is applied locally in muscular pain and inflammation and internally it is used as a carminative in anorexia, insomnia and obstruction in liver and spleen⁷. *Clerodendrum indicum* (L.) Kuntze is mainly used in the treatment of asthma, bronchitis and rheumatism⁸. *Acorus calamus* L. is widely used as an anti-inflammatory agent in the Indian systems of medicine such as *Ayurveda*, *Siddha* and *Unani* for the treatment of various ailments like nervous disorders, bronchitis, digestive disorders, rheumatism, inflammation, depression, tumors, hemorrhoids, skin diseases, general debility and vascular disorders^{9,10}. Considering the medicinal properties and traditional usage of *P. longum*, *C. indicum* and *A. calamus* in rheumatism have prompted us to formulate a polyherbal compound with these plant extracts and study their efficacy or synergistic activity, to minimize individual doses. Following a preliminary screening with different proportions of the extracts, the polyherbal formulation (PHF) in the ratio 3:1:1 (*P. longum*: *C. indicum*: *A. calamus*) was proven effective. Another proposed objective was to study the role of *Piper longum*, whether it increases the bioavailability of the other components as reported in many studies¹¹. Freund's complete adjuvant (FCA)-induced arthritis is the most commonly used animal model for studying its pathogenesis and evaluating anti-arthritic properties of novel compounds. The present study was consequently designed to investigate the anti-inflammatory and anti-arthritic potential of the PHF in FCA- induced arthritis.

Methodology

Drugs and chemicals

FCA, prednisolone, 2-thiobarbituric acid (TBA), trichloroacetic acid, reduced glutathione (GSH), Griess reagent and bovine serum albumin were obtained from Sigma Chemical Co. (St. Louis, Mo,

USA). ELISA kits for estimation of TNF- α , IL-1 β and IL-6 was procured from Ray Biotech, Inc. (Norcross, GA, USA). Revert Aid first strand cDNA synthesis kit was purchased from Thermo Fischer Scientific (MA, USA). All other chemicals and reagents used were of analytical grade procured from Himedia Pvt. Ltd. (Mumbai, India).

Collection of plant material and preparation of extracts

Seeds of *P. longum*, and whole plants of *A. calamus* and *C. indicum* were collected; shade dried and was ground to a fine powder. For preparation of the extracts, 250 gm of the powdered seeds/ plants were soaked in sufficient amount of ethanol (100 % for ethanol extract of *P. longum* and *A. calamus*, 80 % for aqueous ethanolic extract of *C. indicum*) for 72 hrs. They were stirred every 18 hrs and filtered with the Whatman filter paper No.1. The process was repeated three times for exhaustive extraction. The filtrate obtained was evaporated under low pressure to dryness at 50–60 °C in a rotary evaporator (Rotavapor, Buchi R-200, Switzerland) and concentrated over a water bath (< 50 °C). Recovery in terms of dry weight percentage of the ethanol extracts of *P. longum*, *A. calamus* and aqueous ethanol extract of *C. indicum* was 9.45 %, 5.02 % and 1.39 %, respectively.

Animals

Wistar albino rats weighing 180-220 gm, were obtained from the animal house facility of the institute. The animals were housed in polypropylene cages and acclimatized for a week under standard conditions of temperature (22 $\pm$ 3 °C) and humidity (50 $\pm$ 10 %) with a 12 hrs light–dark cycle. The animals had free access to a standard pellet diet and water *ad libitum*. All the protocols were approved by Institutional Animal Ethical Committee (IAEC) of College of Veterinary Sciences, Assam Agricultural University (770/ac/CPCSEA/FVSc, AAU/IAEC/11-12/118). Experimental procedures were performed in accordance with the guidelines recommended by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Experimental design

Experimental animals were randomly divided into five groups, each comprising six animals.

Group 1: Normal control group and received only vehicle.

Group 2: Arthritis control group treated with vehicle.

Group 3: Arthritic rats treated with standard drug PDL (Prednisolone) by oral gavage at a dose of 5 mg/kg b.wt.

Group 4: Arthritic rats treated with PHF by oral gavage at a dose of 100 mg/kg b.wt.

Group 5: Arthritic rats treated with PHF by oral gavage at a dose of 200 mg/kg b.wt.

Induction of arthritis

Arthritis was induced by injection of 0.1 ml of complete Freund's adjuvant into the sub plantar region of the left hind paw of each rat. Each ml of complete Freund's adjuvant contains 10 mg of *Mycobacterium tuberculosis* heat killed and dried in 0.85 ml paraffin oil and 0.15 ml mannide mono oleate. Development of arthritis was monitored by measuring the paw volume using a digital Plethysmometer (520 MR, IITC life Sciences) periodically.

Drug treatment and assessment

All the drug suspensions were freshly prepared in a vehicle containing 0.5 % Carboxymethyl cellulose (CMC), 0.9 % sodium chloride and distilled water at the time of administration. Thirty minutes after oral administration of vehicle or drug, except Group 1, the rats were injected FCA (0.1 ml subcutaneously) in the planter surface of the left hind paw with a 26 gauge needle. This was designated as day 1, and vehicle or drug treatment was continued for 28 days. The dosage of PHF and standard drug PDL used in this study was selected based on our preliminary study and previous report⁹. On day 29, animals were anaesthetized with ether and blood was collected in centrifuge tubes, centrifuged at 3,000 rpm for 10 min and the serum was stored at -80 °C in a deep freezer until the assay. The animals were sacrificed; ankle joints were collected and processed for biochemical, histological and gene expression studies. The paw tissues were immediately dissected out and homogenized in ice-cold phosphate buffer (0.1M, pH 7.4) to give 10 % homogenate, was centrifuged at 10,000 rpm for 10 min and the supernatant obtained was used for biochemical analysis.

Assessment of paw volume

The hind paw volume (HPV) of all animal groups was measured on days 1, 7, 14, 21, and 28 to

determine the severity of paw swelling and deformation after the injection with FCA. The arthritis induced legs of experimental rats were examined and percentage of inhibition was calculated¹².

$$\% \text{ inhibition} = \frac{(C_t - C_0)_{\text{control}} - (C_t - C_0)_{\text{treated}}}{(C_t - C_0)_{\text{treated}}}$$

Assessment of oxidative stress and antioxidant status

Lipid peroxidation and nitric oxide

The LPO end product malondialdehyde (MDA) was estimated in the joint homogenate by Ohkawa *et al.*¹³ method using the thiobarbituric acid and the absorbance was measured spectrophotometrically at 532 nm. The values are expressed as η M of MDA/mg of protein. Nitrite, an indicator of the production of nitric oxide (NO) was determined with a colorimetric assay using Griess reagent (Sigma Aldrich). The concentration of nitrite was determined from a sodium nitrite standard curve and expressed as μ M of nitrite/mg of protein.

Antioxidant status

GSH was estimated according to the method described by Ellman *et al.*¹⁴. The concentration of reduced glutathione was expressed as μ M of GSH/mg protein. SOD activity was estimated using SOD assay kit (Sigma-Aldrich, USA) according to the manufacturer specifications. The SOD activity (units/mg of protein) was calculated by using the standard plot. The CAT activity was determined according to the method of Sinha *et al.*¹⁵. CAT activity was expressed as μ M of H₂O₂ decomposed/min/mg protein. The total protein was estimated by the method of Bradford *et al.*¹⁶.

Estimation of pro-inflammatory cytokines

The levels of pro-inflammatory cytokines (TNF- α , IL-1 β and IL-6) in the serum were estimated using commercially available cytokine ELISA kits (Ray Biotech, USA) according to the manufacturer's instructions.

Histology

Animals were euthanized on day 29 following induction of arthritis. The left knee joints were removed, trimmed, fixed in 10 % formalin and then decalcified in 10 % EDTA for 2 weeks¹⁷. The decalcified joints were then dehydrated by processing

with different grades of alcohol and chloroform mixture. The joint tissues were embedded in paraffin and sliced (5 μ m) for the histological examination. The sections were stained with hematoxylin and eosin dye for morphological evaluation, observed under microscope and photographed.

RT-PCR analysis

Reverse transcriptase-polymerase chain reaction was performed following the methods used by Bodduluru *et al.*¹⁸. cDNA was synthesized using Revert Aid first strand cDNA synthesis kit (Thermo Scientific) according to the manufacturer's instructions. Oligonucleotide primers used for amplification were shown in Table 1. All PCR samples were denatured at 95°C for 3 min before cycling and were extended for 10 min at 72°C after cycling. The PCR assay using primers was performed for 30 cycles at 95 °C for 30s, annealing temperature varies for different primers for 45s (Table 1) and 72°C for 45s (Veriti Thermal Cycler, Applied Biosystems). GAPDH served as internal control to check for equal loading. PCR products were analyzed using the Image Lab 5.1 (Bio Rad Co).

Statistical analysis

The statistical analysis was performed using GraphPad Prism software version 5.0 (San Diego, CA, USA). The statistical significance of differences among various experimental groups was calculated by one-way ANOVA followed by Tukey's post hoc analysis. All the values are expressed as mean $\pm$ S.D. Results were considered statistically significant when $p < 0.05$.

Results

Effect of PHF on rat HPV

The hind paw volume (HPV) was determined to assess the extent of adjuvant arthritis induced by FCA. As shown in Table 2, a significant ($p < 0.001$) increase in HPV was observed in adjuvant-treated group on the 7th, 14th, 21th and 28th day as compared with control group. However, treatment with PDL (5 mg/kg) and PHF (100 and 200 mg/kg) significantly ($p < 0.001$) reduced the paw edema volume in both the injected and non-injected paw throughout the experiment in contrast with the FCA control (arthritis group).

Table 1—Oligonucleotide primer sequences for target genes used in RT-PCR.

Gene of interest	Primer sequences	Annealing temperature	Product size
NF κ B-p65	FP: 5'-GCTTTGCAAACCTGGGAATA-3' RP: 5'-CAAGGTCAGAATGCACCAGA-3'	60°C	123bp
COX2	FP: 5'-CTGAGGGGTTACCACTTCCA-3' RP: 5'-TGAGCAAGTCCGTGTTCAAG-3'	62°C	209bp
iNOS	FP: 5'-CACCCGAGATGGTCAGGG-3' RP: 5'-CCACTGACACTCCGCACAA-3'	59°C	105bp
GAPDH	FP: 5'-AGGTTGTCTCCTGTGACTTC-3' RP: 5'-CTGTTGCTGTAGCCATATTC-3'	58°C	130bp

Table 2—Inhibition of left and right hind paws edema by polyherbal formulation on adjuvant-induced arthritis in rats. Values represent the mean $\pm$ S.D. of six animals for each group. Each value in parenthesis indicates the percentage inhibition rate. ^{###} $p < 0.001$ compared with normal control. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared with arthritic control.

Days	Normal control	Arthritis control	FCA+ PDL (5 mg/kg)	FCA+PHF (100 mg/kg)	FCA+PHF (200 mg/kg)
Edema volume of left hind paw (mL)					
7	0.08 $\pm$ 0.02	1.02 $\pm$ 0.08 ^{###}	0.75 $\pm$ 0.09 (26.47)***	0.97 $\pm$ 0.08 (4.90)	0.81 $\pm$ 0.07 (20.58)***
14	0.27 $\pm$ 0.06	1.15 $\pm$ 0.17 ^{###}	0.73 $\pm$ 0.06 (36.52)***	1.01 $\pm$ 0.11 (12.17)	0.83 $\pm$ 0.03 (27.82)***
21	0.20 $\pm$ 0.04	1.28 $\pm$ 0.13 ^{###}	0.50 $\pm$ 0.05 (60.93)***	0.90 $\pm$ 0.10 (29.68)***	0.71 $\pm$ 0.05 (44.53)***
28	0.17 $\pm$ 0.01	1.23 $\pm$ 0.14 ^{###}	0.37 $\pm$ 0.03 (69.91)***	0.74 $\pm$ 0.07 (39.83)***	0.57 $\pm$ 0.02 (53.65)***
Edema volume of right hind paw (mL)					
7	0.06 $\pm$ 0.10	0.57 $\pm$ 0.14 ^{###}	0.27 $\pm$ 0.14 (52.63)***	0.42 $\pm$ 0.13 (26.13)***	0.34 $\pm$ 0.12 (40.35)***
14	0.12 $\pm$ 0.07	0.79 $\pm$ 0.17 ^{###}	0.24 $\pm$ 0.11 (69.6)***	0.49 $\pm$ 0.12 (37.97)***	0.38 $\pm$ 0.07 (51.89)***
21	0.1 $\pm$ 0.11	0.83 $\pm$ 0.12 ^{###}	0.18 $\pm$ 0.11 (78.3)***	0.37 $\pm$ 0.09 (55.42)***	0.27 $\pm$ 0.10 (67.46)***
28	0.08 $\pm$ 0.13	0.80 $\pm$ 0.11 ^{###}	0.15 $\pm$ 0.13 (81.25)***	0.33 $\pm$ 0.12 (58.75)***	0.24 $\pm$ 0.14 (70.00)***

Effect of PHF on oxidative Stress and antioxidant status

We estimated various pro-oxidant markers (MDA and NO) and antioxidant proteins (GSH, SOD and CAT) in the paw tissues of control and experimental groups. As shown in Fig. 1, the LPO and NO levels were significantly ($p < 0.001$) increased in the paw tissues of arthritic rats as compared to the control group. Treatment with PHF significantly ($p < 0.001$) decreased LPO and NO levels in the paw tissues of arthritic rats similar to PDL. Our results also revealed substantial reduction in antioxidant status (GSH, SOD and CAT) in the joint homogenate of arthritic rats when compared to normal controls (Fig. 2). However, treatment with PDL and PHF significantly ($p < 0.001$) prevented the oxidative stress by increasing the antioxidant status in the joint homogenate of arthritic rats as compared to FCA Control. These findings demonstrated the protective activity of PDL and PHF on oxidative stress in FCA-induced animals.

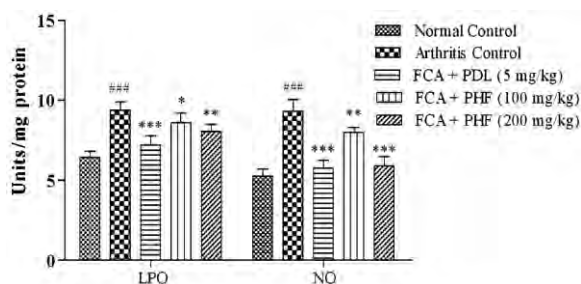


Fig. 1—Effect of polyherbal formulation and prednisolone on lipid peroxidation and nitric oxide levels in the paw tissues of control and experimental rats. All the values are expressed as mean $\pm$ S.D. for six rats in each group. ### $p < 0.001$ compared with normal control. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared with arthritic control. Units: LPO- η M of MDA released/ mg protein; NO- μ M of nitrite/ mg protein.

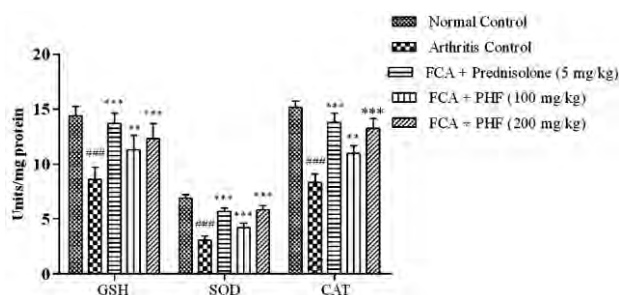


Fig. 2—Effect of polyherbal formulation and prednisolone on antioxidants status in the paw tissues of control and experimental rats. All the values are expressed as mean $\pm$ SD of six animals. ### $p < 0.001$ compared with normal control. ** $p < 0.01$; *** $p < 0.001$ compared with arthritic control. Units: GSH- μ M of GSH/mg protein; SOD-Units/mg protein; CAT- μ M of H_2O_2 consumed/min/mg protein.

Effect of PHF on inflammatory cytokines

To investigate the anti-inflammatory effect of PHF the serum levels of proinflammatory cytokines (TNF- α , IL-1 β and IL-6) in control and experimental rats were measured by ELISA. As depicted in Fig. 3, the levels of pro-inflammatory cytokines (TNF- α , IL-1 β and IL-6) in serum were significantly increased in arthritic rats as compared with control rats. On the contrary, arthritic rats treated with PDL (5 mg/kg) and PHF (100 and 200 mg/kg) showed significant reduction in the levels of pro-inflammatory cytokines when compared to arthritic control rats indicating potent inhibitory effects on the production of TNF- α , IL-1 β and IL-6.

Effect of PHF on joint histology

In the present study, the histopathological analysis of the joints samples from control group showed normal joint architecture and cell structure (Fig. 4A). The joints from arthritic rats showed an abnormal joint architecture with increased synovial hyperplasia, massive inflammatory cell infiltration, pannus formation and bone erosion as compared to normal control (Fig. 4B). In contrast, the PDL administered group showed a marked decrease in synovial inflammatory cell infiltration and synovial hyperplasia with moderate obliteration of pannus formation and bone erosion (Fig. 4C). Similarly, the PHF (100 and 200 mg/kg) administered group showed decreased synovial hyperplasia, inflammatory cell infiltration and pannus formation compared with the arthritic control group (Figs. 4D&E). These findings showed the evidence of PHF and PDL in suppressing chronic inflammation and bone destruction of in the arthritis rats.

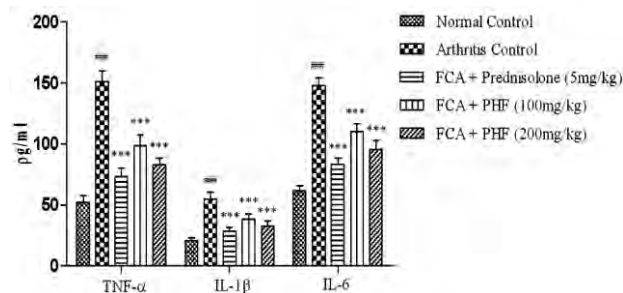


Fig. 3—Effect of polyherbal formulation and prednisolone on serum proinflammatory cytokines of control and experimental rats. All the values are expressed as mean $\pm$ SD of six animals. ### $p < 0.001$ compared with normal control. *** $p < 0.001$ compared with arthritic control.

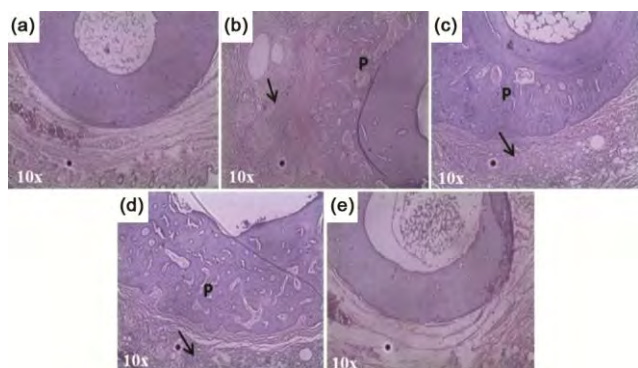


Fig. 4—Histopathological changes in paw tissue sections of control and experimental animals (A) Control animal showing intact articular cartilage with no evidence of inflammation; (B) Arthritic induced animal showing pannus formation (P), synovial hyperplasia and intense infiltration of inflammatory cells (indicated by arrow); (C) Prednisolone (5 mg/kg b.wt)-treated animal showing moderate inflammation and reduced amount of immune cell infiltration (arrow). (D) PHF (100 mg/kg b.wt)-treated animal showing intact articular cartilage and less infiltration of immune cells (arrow); (E) PHF (200 mg/kg b.wt)-treated animal showing intact articular cartilage and less evidence of inflammation and infiltration of immune cells.

Effects of PHF on COX-2, iNOS and NF- κ B gene expression

To investigate the molecular mechanism of anti-inflammatory effect of PHF and PDL in arthritic rats, we examined the mRNA expression levels of inflammatory enzymes (COX-2 and iNOS) and transcription factor (NF- κ Bp65) in the joint homogenate of arthritic rats. The mRNA expression levels of inflammatory marker enzymes (COX-2 and iNOS), and transcription factor (NF- κ B-p65) were significantly upregulated in the paw tissues of arthritic rats compared to the control group (Fig. 5). However, treatment with PHF significantly down regulated the mRNA expression of these genes in the paw tissues of arthritic rats similar to that of PDL when compared with arthritic control rats.

Discussion

Herbal medicines with high efficacy and fewer side effects have become more popular and well explored in recent decades¹⁹. *Piper longum*, *Clerodendrum indicum* and *Acorus calamus* were used in traditional Indian medicine for the treatment of rheumatism and inflammation of joints. However, the anti-inflammatory and anti-arthritic activities have been poorly explored. Therefore, the present study aimed to investigate the anti-inflammatory and anti-arthritic effects of PHF comprising of the standardized extracts of *Piper longum*, *Clerodendrum indicum* and *Acorus*

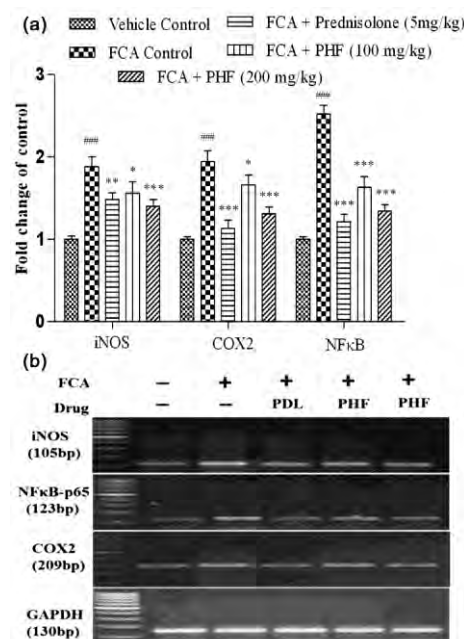


Fig. 5—Effect of polyherbal formulation and prednisolone on iNOS, COX2 and NF- κ B mRNA expression in the paw tissues of control and experimental animals. (A) Quantitative data expressing the corresponding mRNA levels were assessed using densitometry and is expressed as ‘fold changes’ as compared with control. Values represent the mean $\pm$ SD (n=3) in each group. Statistical significance: ###p < 0.001 compared with normal control. *p < 0.05; **p < 0.01; ***p < 0.001 compared with arthritic control. (B) Lane 1-DNA marker, Lane 2-Normal control, Lane 3-Arthritis control, Lane 4- FCA+PDL, Lane5-FCA+PHF (100 mg/kg), Lane 6-FCA+PHF (200 mg/kg). GAPDH was used as a positive control to assess the equal loading of sample.

calamus and explore the possible molecular mechanism in adjuvant-induced arthritic rats. The study demonstrated that PHF has the potential to suppress the various aspects of inflammatory immune responses and molecular events in adjuvant-induced arthritis by modulating antioxidants and pro-inflammatory cytokines, and attenuating the expression of pro-inflammatory mediators and transcription factor (NF κ B) in the synovial joint.

In the present study, arthritis was induced by sub planter injections of FCA into the footpad of the left hind paw. The increase in the paw volumes in both FCA-injected and non- FCA-injected paws indicates the primary and secondary arthritic lesions respectively. Administration of PDL and PHF significantly suppressed the paw swelling induced by the FCA. The significant reductions of the primary and secondary lesions by these drugs indicate the possible anti-inflammatory and immunosuppressive activity, respectively²⁰. The histopathological

observations also correlated with these parameters, which further support the anti-inflammatory effect of PDL and PHF. Reactive oxygen species (ROS) are apparently involved in a wide variety of pathologies, including rheumatoid arthritis, malignant diseases, atherosclerosis and several chronic inflammatory processes²¹. In this study, elevated lipid peroxidation and nitric oxide levels, the well known markers of oxidative stress were observed in arthritic rats as compared to normal rats. However, PHF decreased lipid peroxidation and NO levels in the paw tissues of arthritic rats towards normal level indicating the deterrence of the cell damage by reducing oxidative stress. GSH is a predominant low molecular weight thiol and acts as an intracellular reductant in oxidation-reduction processes. It is the first line of defense against damage caused by ROS and organic peroxides²². SOD and CAT play an important role in the detoxification of superoxide anion and hydrogen peroxide respectively, and protects the cells against oxidative damage induced by free radicals¹⁹. The decreased levels of the antioxidants might be due to the excessive consumption to defend oxidative damage and/ or inactivation or inhibition of the enzymes by hydrogen peroxide²³. Treatment with PDL and PHF significantly blunted the depletion of GSH, SOD and CAT, probably by competing for scavenging of free radicals, and preserving the integrity of cellular membranes.

The pro-inflammatory cytokines, TNF- α , IL-1 β , and IL-6 produced by the activated immune cells are important initiators of the inflammatory response, involved in the pathogenesis of joint inflammation and cartilage degradation during RA²⁴. In this study, the levels of TNF- α , IL-1 β and IL-6 were significantly elevated in arthritis control animals. Administration of PHF markedly attenuated serum levels of TNF- α , IL-1 β and IL-6 indicating the disease modifying potential of PHF. Accumulating evidence supports that NF- κ B plays substantial role in the pathogenesis of RA²⁵. The transcription of proinflammatory cytokines, mediators, chemokines, enzymes, and adhesion molecules has been shown to be dependent on NF- κ B activation²⁵. The pro-inflammatory mediator, COX-2 plays a crucial inflammatory role in RA and the expression of COX-2 is associated with the generation of PGE2 that causes vasodilation and recruitment of neutrophils to the arthritic joints²⁶. iNOS is a major inflammatory enzyme that promotes the production of NO via activation of the iNOS pathway. Literature reports

that NO plays a role in severe joint inflammation and tissue damage that contribute to the disease progression via production of TNF- α , IL-1 β , INF γ , as well as MMPs on RA synovium²⁷. In the present study, elevated expression of COX2, iNOS and NF- κ B mRNA were observed in the paw tissues of arthritic rats. Interestingly, we found that the administration of PHF and PDL markedly down regulated the mRNA expression of COX2, iNOS and the transcription factor NF- κ B in the paw tissues of arthritic rats. These observations suggest that the suppressive effect of PHF on pro-inflammatory cytokines, inflammatory mediators and enzymes could be due to its inhibitory action on NF- κ B signaling pathway.

Conclusion

In conclusion, our study demonstrated that PHF was effective in FCA induced arthritis in rats, as evidenced from the reduced hind paw volume and amelioration of bone and cartilage degradation. The anti-arthritic activity of PHF could be attributed to its ability of decreasing the levels of proinflammatory cytokines (TNF- α , IL-1 β and IL-6), altering the oxidant/antioxidant balance as well as down-regulating the inflammatory marker enzymes COX2, iNOS and transcription factor NF- κ B. Taken together, these finding suggest that PHF has the potential to relieve clinical symptoms and to improve quality of life of arthritic patients, and can be used as an anti-arthritic drug for the treatment of RA.

Conflict of Interest

The authors declare no conflicts of interest.

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