Effect of ethanol extract of an ayurvedic preparation (Pathyadya Churna) on arthritis in rats

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ABSTRACT

Objectives: To study the anti-arthritic activity of Pathyadya Churna ethanol extract (PCE) in rats.

Materials and Methods: Formaldehyde (2% v/v) or complete Freund’s adjuvant (CFA 0.1 mL) was injected in the left hind paw of male Wistar rats to develop arthritis. These rats were treated with three doses (135, 270, and 540 mg/kg) of PCE and one dose (10 mg/kg) of indomethacin. Anti-arthritic activity of the extract was assessed by noting paw volumes, rheumatoid factor (RF), blood parameters, and histological changes.

Results: PCE treatment reduced paw swelling in arthritis caused by both formaldehyde and CFA. In CFA-treated rats, a significant decrease (P < 0.001) was seen in hemoglobin (13.92 g/dL to 9.97 g/dL), red blood cell count (7.32 million/mm³ to 6.58 million/mm³), and packed cell volume (44.04% to 30.56%). There were also significant (P < 0.001) elevations in white blood cell count (8220–11,420/mm³), platelets (2.46–4.15 lakhs/mL), erythrocyte sedimentation rate (3.76–8.03/60 min), RF (7.17–26.77 IU/mL), triglycerides (71.69–96.60 mg/dL), total cholesterol (96.85–145.05 mg/dL), low-density lipoprotein (53.11–109.60 mg/dL), and very low-density lipoprotein (14.34–19.32 mg/dL). In CFA-induced arthritic rats, high-density lipoprotein decreased significantly (29.40 mg/dL to 16.13 mg/dL). Marked changes were noted in the histology of ankles. Treatment with PCE significantly reversed all these hematological and histological changes in a dose-dependent manner.

Conclusions: PCE has a significant anti-arthritic activity in rats and is free from toxic effects.

KEY WORDS: Complete Freund’s adjuvant, formaldehyde, hematological parameters, histology of ankle joint, Pathyadya Churna

Introduction

Rheumatoid arthritis (RA) is a chronic disease of the joints mediated by immunological pathways leading to joint inflammation and degeneration. A wide variety of triggers are involved in the initiation and progression of the disease resulting in damage of the synovial and cartilage cells. These include genetic predisposition, rheumatoid factor (RF), complement activation, different kinins, metabolites of arachidonic acid, and lymphocytes. RF is a classic autoantibody in RA.

Currently, nonsteroidal anti-inflammatory drugs, disease-modifying anti-rheumatic drugs, tumor necrosis factor alpha (TNF-α) inhibitors, corticosteroids, etc., are given for the treatment of RA. These therapies of RA, on long-term use, lead to some side effects such as peptic ulcer and...
bleeding, depression, bone marrow suppression, diarrhea, edema, dizziness, and renal insufficiency. Plant-based complementary and alternative medicines are also being used by arthritic patients to get relief from this debilitating disease.

The description of Amavata in Ayurveda finds many similarities with many of the rheumatic diseases. It is said that a person having inherent suboptimal digestive capacity develops ama when exposed to incompatible diet and physical activities. Ama escapes into the circulation and gets further vitiated. The vitiated dosha and Ama take shelter in Sandhi Sthana and derange the shleha kapha, especially in joints. The patients get symptoms such as sandhi shula, sandhi shotha, sandhi graha, and sparshashatwa, which are very much akin to the symptoms of RA. Such vitiated ama gets deposited in various tissues and leads to disease. The therapy in Ayurveda, aims at increasing the digestive power, removal of metabolic and other toxins, balancing the immune system, minimizing pain, swelling, and deformities.

Pathyadya Churna is a classical Ayurvedic preparation, which consists of dried rhizome of Sunthi (Zingiber officinale Roxb., Zingiberaceae), fine powders of dried pericarp of Haritaki (Terminalia chebula Retz., Combretaceae), and dried fruits of Ajwain (Trachyspermum ammi Linn., Umbelliferae) in equal quantities. It is recommended for treating rheumatism, inflammation, indigestion, and heart diseases. However, there is no scientific study on the effect of this important formulation on arthritis. Hence, we studied the effect of Pathyadya Churna ethanol extract (PCE) on two arthritic models in rats using formaldehyde and complete Freund’s adjuvant (CFA). Changes in blood parameters, RF levels, and histology of ankles were also evaluated.

Materials and Methods

Herbal Drugs and Chemicals

Pericarp pieces of Haritaki, rhizome of Sunthi, and cremocarp of Ajwain were purchased from M/s. Yucca Enterprises, Mumbai, Maharashtra, India. A qualified botanist authenticated the herbs by morphological and microscopic studies. The samples were also compared with the standard herbarium specimens deposited in the first author’s department. These herbal drugs were powdered to 80# and stored in a dry cool place away from light. Powders of the three herbs were mixed thoroughly in equal proportions.

Standard analytical grade reagents, chemicals, and CFA (Sigma Aldrich, USA) were used in all the experiments.

Extraction of Pathyadya Churna with Ethyl Alcohol

PCE was prepared by soaking 50 g of the powder formulation in 300 mL ethanol for 24 h. The extract was filtered and concentrated under reduced pressure on a rotary evaporator (Equitron Roteva, Medical Instrument Manufacturing Company., Mumbai, Maharashtra, India) and dried completely in a vacuum desiccator to a semisolid mass.

Animals

Male albino rats (Wistar) weighing 200–250 g available from the institute’s animal house and maintained in standard conditions (25 ± 2°C temperature, 75 ± 5% relative humidity, and 12 h light-dark cycle) were used for the study. All the animals had free access to standard pellet diet (M/s. Hindustan Lever Ltd., Bengaluru, Karnataka, India) and distilled water. Prior approvals of the protocols from Institutional Animal Ethical Committee before the start of the experiments were obtained (Approval Numbers: PIPH 06/12 and PIPH 36/13).

Acute Toxicity Study

OECD guidelines (2001) were followed for evaluating acute toxicity of PCE. The powder formulation was suspended in 0.5% gum acacia and given to overnight fasted healthy rats (n = 6) at a dose of 2000 mg/kg (p.o.). Gross behavior of the rats was continuously monitored for 24 h. The animals were also observed for any mortality in the next 14 days.

Formaldehyde-Induced Arthritis

Animals were randomly assigned to six groups with six rats in each group. The drug administration to each group is as follows:

- Group I: 0.5% (p.o.) gum acacia suspension in water (normal control)
- Group II: 0.1 mL (sub-plantar) 2% v/v formaldehyde (arthritic control)
- Group III: 10 mg/kg (p.o.) indomethacin (standard control)
- Group IV: 135 mg/kg (p.o.) PCE suspended in gum acacia (treatment)
- Group V: 270 mg/kg (p.o.) PCE suspended in gum acacia (treatment)
- Group VI: 540 mg/kg (p.o.) PCE suspended in gum acacia (treatment)

Thirty minutes after the administration of the vehicle/drug, arthritis was induced by sub-plantar administration of 0.1 mL formaldehyde (2% v/v) into the left hind paw of all the animals on day 0 and 3. Indomethacin and the test drug were given up to day 10.

Paw Volume in Formaldehyde-Induced Arthritis

Volumes of the formaldehyde-injected paws of all the rats were noted on a Digital Plethysmometer (Orchid Scientific and Innovative India Pvt. Ltd., Maharashtra, India) on days 0, 2, 4, 6, 8, and 10. Increase in the volume over that of day 0 was taken as net inflammation.

Complete Freund’s Adjuvant Arthritis in Rats

Details of animal grouping and treatment doses are same as described above excepting that the disease control group (Group II) was given a single injection of 0.1 mL CFA in place of formaldehyde in the sub-plantar region of the left hind paw. Rats were left for 14 days after the injection of CFA to allow the development of arthritis. Starting from day 14, indomethacin and PCE were administered (p.o.) daily until day 28. CFA produced significant swelling and redness in paw over 24 h of CFA challenge and led to the production of chronic inflammation. Body weights and paw volumes of all animals were recorded at regular intervals from day 14 to day 28. After recording these parameters on day 28, rats were sacrificed by cervical decapitation after collecting blood. Ankle joints were severed and processed for histological studies.
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Paw Volume and Body Weight in Complete Freund’s Adjuvant Induced-Arthritis

Volumes of the left hind paw in all the animals were recorded on digital plethysmometer before injecting CFA injection on day 0. Starting from day 14, paw volumes were noted at regular intervals until day 28. Before noting the paw volumes, body weights of animals were also taken on a balance used for weighing small animals. Percent reduction in inflammation was derived from the following formula:

\[
\% \text{ anti-inflammatory activity} = 100 - \left( \frac{\text{increase in paw volume from day 0 to day 28}}{\text{increase in paw volume from day 0 to day 14}} \right)
\]

Analysis of Blood for Various Parameters in Complete Freund’s Adjuvant-Induced Arthritis

On day 28, blood was collected from retro-orbital plexus of animals of all the groups. Using a HEMA2062 Hematology Cell Counter (Analytical Technologies Limited, India), hemoglobin (Hb), red blood cell (RBC), white blood cell (WBC), packed cell volume (PCV), platelet, and erythrocyte sedimentation rate (ESR) were analyzed. RF TurbilatexTL020 (Chemelex, S.A, Barcelona) was used to determine RF. A semi-auto analyzer photometer 5010 (Piramal Healthcare V3.1, Mumbai, Maharashtra, India) was employed for the determination of various lipids in the serum. Cholesterol-LS kit and Triglycerides (TG)-LS kit (Piramal Enterprises Ltd., Navi Mumbai, Maharashtra, India) were employed for the analysis of total cholesterol (TC) and total TG. High-density lipoprotein (HDL) Direct kit (Erba Lachema s.r.o., Karasek, CZ) was used to analyze HDL-cholesterol. Low-density lipoprotein-cholesterol (LDL) and very low-density lipoprotein-cholesterol (VLDL) were calculated from TC, TG, and HDL.

Histological Analysis

The severed ankle joints of left hind paws from animals of all groups in CFA-induced arthritis were fixed in 10% buffered formalin for 24 h. After passing through alcohol-xylene series and embedding in paraffin, 5 µm thick sections were taken on a rotary microtome RM 2125 RTS (Leica Microsystems Incorporation, USA). After staining with hematoxylin and eosin,[16] the sections were observed under light microscope and changes in cellular architecture were noted.

Data Analysis by Statistical Methods

GraphPad Prism version 5.03 software (GraphPad Software, Inc., U.S.A) was used. Data were subjected to two-way ANOVA followed by Bonferroni test for multiple comparisons. \( P < 0.05 \) was taken as significant difference.

Results

Acute Toxicity

On administration of PCE at 2000 mg/kg, neither mortality nor any changes in the autonomic and behavioral patterns of animals indicating any toxicity were noted till 14 days and thereafter.

Effect of Pathyadya Churna Ethanol Extract on Paw Volume in Formaldehyde-Induced Arthritis

Injection of formaldehyde led to joint swelling in all the animals. A significant reduction in swelling was noted in animals treated with PCE and indomethacin. The response was dose dependent [Figure 1]. Rats treated with 270 mg/kg and 540 mg/kg PCE (Group V and VI, respectively) exhibited higher activity.

Changes in the Inflammation of Paw and Body Weight of Complete Freund’s Adjuvant Arthritic Rats Treated with Pathyadya Churna Ethanolic Extract

CFA produced clear and significant inflammation of the paw, and maximum swelling was observed on day 14 after injecting CFA. PCE (135 mg/kg, 270 mg/kg, and 540 mg/kg) and indomethacin (10 mg/kg) were administered every day starting from day 14 until day 28. PCE treatment significantly \( (P < 0.001) \) reduced the swelling of the paw in a dose-dependent manner [Figure 2]. On day 28, there was 87.32% and 90.14% reduction in joint swelling in rats treated with 270 mg/kg and 540 mg/kg PCE, respectively. There were no noticeable differences in the body weights of the rats from all the groups.

Effect of Pathyadya Churna Ethanol Extract on Various Blood Parameters in Complete Freund’s Adjuvant Arthritic Rats

There was decrease in the levels of Hb from 13.92 to 9.97 g/dL, RBC from 7.32 to 6.58 million/mm³, and PCV from 44.04% to 30.56% in arthritic rats with concurrent increase in WBC from 8.22 to 11.42 thousands/mm³, platelet count from 2.46 to 4.15 lakhs/mL, ESR from 3.76 to 8.03/60 min, and RF from 7.17 to 26.77 IU/mL. Significant decrease in HDL from 29.40 to 16.13 mg/dL, increase in TC from 96.85 to 145.05 mg/dL, TG from 71.69 to 96.60 mg/dL, LDL from 53.11 to 109.60 mg/dL, and VLDL from 20.60 to 29.40 mg/dL were observed. PCE brought significant \( (P < 0.001) \) and dose-dependent improvements in all these parameters. On treatment with PCE, various blood parameters returned to near normal levels in arthritic rats [Table 1].

Changes in the Histology of Ankle Joint after Pathyadya Churna Ethanol Extract Treatment

Arthritic joints of the disease control rats in Group II showed edematous inflammation, vascularity due to vasodilatation, clear infiltration of mononuclear inflammatory cells, synovial hyperplasia, and occasional formation of lymphoid follicles. Such changes were observed to be much less in the rats treated with PCE at 270 and 540 mg/kg doses. The integrity of the synovial membrane was found to be more intact as compared to that in the disease control group [Figure 3].

![Figure 1: Effect of Pathyadya Churna ethanol extract on paw volume of formaldehyde-treated animals. Values are expressed as mean ± standard error of mean (n = 6). Statistical analysis by two-way ANOVA followed by Bonferroni test for multiple comparisons](image-url)
Discussion

Acute toxicity study showed the PCE to be free from any toxic effects. Anti-arthritic activity of this extract was studied in two models of arthritis in rats by injecting formaldehyde and CFA. The effect of PCE on the changes in hematological and lipid profiles was also studied.

Ethanol extract of this classical ayurvedic formulation of Pathyadya Churna reduced joint swelling in both the arthritic models. Maximum activity was found at a dose of 540 mg/kg. Arthritis induced by CFA shows close similarities in many clinical and immunological aspects of human arthritis.\(^{[15]}\) In CFA-induced arthritis, the effect of PCE at 540 mg/kg was found to be better than indomethacin.

Changes in hematological parameters were observed due to RA. In RA, cytokines such as TNF-α and interleukin-6 (IL-6) originated in the synovial tissues are released into the systemic circulation leading to systemic inflammation.\(^{[15]}\) This is reflected in endothelial dysfunction, insulin resistance, lipid abnormality, pro-thrombotic, and pro-oxidative effects.\(^{[18]}\)

The decrease in Hb and RBC is indicative of anemia in rats due to endothelial dysfunction.\(^{[19,20]}\) Pathogenic micro-organisms invading the body stimulate the immune system resulting in increased RF, WBC, and platelet counts.\(^{[21]}\) In chronic conditions of arthritis, ESR increases drastically. The higher levels of TG and LDL are mediated by an enhanced release of TNF-α, which stimulates LDL production.\(^{[22]}\)

Ginger, one of the ingredients of Pathyadya Churna, is recommended in Ayurveda for the treatment of Amavata, which is akin to arthritis of modern medicine. There are reports where the beneficial effects of ginger in RA were ascribed to the inhibition of both the cyclooxygenase enzymes 1 and 2. This, in turn, is responsible for the reduction of prostaglandins and leukotrienes.\(^{[23,24]}\) Aqueous alcohol extract of T. chebula pericarp powder was reported to inhibit the expression of TNF-R1, IL-6, and IL-1 β in the synovium of CFA-induced arthritis and reduce serum TNF-α level.\(^{[25,26]}\) Thangam and Dhananjayan reported that total alcoholic and aqueous extracts of ajwain exhibited significant anti-inflammatory and anti-arthritic effect in rats.\(^{[27]}\)

It is unclear whether the adverse changes in the various blood parameters, lipid levels, and cellular architecture in the ankle joints occurred simultaneously along with the progression of inflammation from day 0 to 14 or at a later stage between day 14 and 28. PCE and indomethacin were given starting from day 14 to 28. As evident from data in Table I, all the blood parameters and different lipids returned to near normal levels when treated with PCE. The ingredients of PCE have a wide variety of phytochemicals such as oleo resins, terpenes, phenols, tannins, and polysaccharides. A synergistic action of all these molecules can be expected to bring about a holistic change by reducing inflammation and arthritis. Inhibition of cytokines by ginger and T. chebula leads to recovery in altered liver function and brings back the normal levels of cholesterol. In addition, the inhibition of cytokines stops the degradation and phagocytosis of erythrocytes and restores the levels of RBC and Hb. This results in the recovery of anemia produced by arthritic conditions. Inhibition of inflammation by PCE is also associated with reversal of increased levels of RF, WBC, and platelets.

Treatment with PCE minimized injury to the cells in the ankle joints. The effect was very prominent with a dose of 540 mg/kg. There was greater increase in synocytes number and decrease in the number of inflammatory cells as compared to even standard indomethacin treatment. The effect was found to be moderate with a lower dose of 135 mg/kg PCE.

Conclusion

Our study thus demonstrates anti-arthritic activity of PCE and justifies the use of Pathyadya Churna in Ayurveda for the treatment of arthritis.

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**Figure 3:** Effect of Pathyadya Churna ethanol extract on the histology of ankle joint in experimental rats in complete Freund’s adjuvant-induced arthritis. Paraffin-embedded sections stained with hematoxylin and eosin.
Table 1:

Effect of *Pathyadya Churna* ethanol extract on hematological parameters and lipid profile in complete Freund’s adjuvant-induced arthritic rats

| Parameters | 0.5% gum acacia vehicle control | 0.1 mL CFA arthritic control | 0.1 mL CFA + indomethacin 10 mg/kg | 0.1 mL CFA + PCE 135 mg/kg | 0.1 mL CFA + PCE 270 mg/kg | 0.1 mL CFA + PCE 540 mg/kg |
|------------|---------------------------------|-----------------------------|------------------------------------|--------------------------|--------------------------|--------------------------|
| Lipids (mg/dL) |                                 |                             |                                    |                          |                          |                          |
| TC         | 96.85±1.33                      | 145.05±0.88                 | 113.85±1.53*                       | 124.34±1.81*            | 115.89±1.73*            | 111.64±1.59*            |
| TG         | 71.69±1.06                      | 96.60±1.20                  | 78.02±0.88*                        | 90.67±0.86*             | 84.51±1.16*             | 81.87±1.21*             |
| HDL        | 29.40±0.33                      | 16.13±0.58                  | 24.67±0.58*                        | 23.18±0.87*             | 27.41±1.53*             | 27.91±0.37*             |
| LDL        | 53.11±1.73                      | 109.60±1.16                 | 73.57±1.20*                        | 83.03±1.27*             | 71.57±1.73*             | 67.36±1.19*             |
| VLDL       | 14.34±0.37                      | 19.32±0.87                  | 15.60±0.48*                        | 18.13±0.72*             | 16.9±0.72*              | 16.37±0.68*             |
| Complete blood count |                             |                             |                                    |                          |                          |                          |
| Hb (g/dL)  | 13.92±1.27                      | 9.97±1.03                   | 13.23±1.43*                        | 11.13±1.18*             | 12.64±1.33*             | 13.28±1.27*             |
| RBC (millions/mm³) | 7.32±0.87                      | 6.58±0.74                   | 7.31±0.91                          | 7.10±0.88               | 7.24±0.88               | 7.31±0.78               |
| WBC (thousands/mm³) | 8.22±0.67                      | 11.42±0.79                  | 8.89±0.74*                         | 9.08±0.89               | 8.80±0.73               | 8.42±0.84*              |
| Platelet (lakhs/mL) | 2.46±0.23                      | 4.15±0.41                   | 2.86±0.33*                         | 3.12±0.41               | 2.87±0.17               | 2.67±0.32*              |
| PCV (%)    | 44.04±1.21                      | 30.56±1.43                  | 36.5±1.50*                         | 31.67±1.76*             | 36.29±1.67*             | 38.42±1.86*             |
| ESR (60 min) | 3.76±0.31                      | 8.03±0.42                   | 3.95±0.47*                         | 6.32±0.54               | 4.84±0.53               | 4.12±0.66*              |
| RF (IU/mL) | 7.17±0.13                       | 26.77±1.46                  | 10.78±0.77*                        | 13.35±0.67*             | 11.55±0.91*             | 11.20±1.01*             |

*a MeansSEM (n=6), *p<0.001. TC=Total cholesterol, TG=Triglycerides, HDL=High-density lipoprotein, LDL=Low-density lipoprotein, VLDL=Very low-density lipoprotein, WBC=White blood cell, RBC=Red blood cell, PCV=Packed cell volume, ESR=Erythrocyte sedimentation rate, RF=Rheumatoid factor, SEM=Standard error of mean, CFA=Complete Freund’s adjuvant, Hb=Hemoglobin

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**Conflicts of Interest**

There are no conflicts of interest.

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