International Immunopharmacology 20 (2014) 124–130

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Polyphenolics isolated from virgin coconut oil inhibits adjuvant induced arthritis in rats through antioxidant and anti-inflammatory action

A. Vysakh [a], M. Ratheesh [b,*], T.P. Rajmohanan [c], C. Pramod [d], S. Premlal [e], B. Girish kumar [f], P.I. Sibi [d]

[a] Department of Applied Microbiology, St. Thomas College, Pala, Kottayam, Kerala, India
[b] Department of Biochemistry, St. Thomas College, Pala, Kottayam, Kerala, India
[c] College of Pharmaceutical Sciences, Government Medical College, Thiruvananthapuram, India
[d] Department of Pharmacology, University College of Pharmacy, MG University, Kottayam, Kerala, India
[e] Drug Standardization Unit, Govt. Ayurveda College, Thiruvananthapuram, Kerala, India
[f] Department of Zoology, MSM College, Kayamkulam, Kerala, India

ARTICLE INFO

Article history:
Received 20 July 2013
Received in revised form 26 January 2014
Accepted 19 February 2014
Available online 6 March 2014

Keywords:
Antioxidant
Anti-inflammatory
Virgin coconut oil
Polyphenols

ABSTRACT

We evaluated the protective efficacy of the polyphenolic fraction from virgin coconut oil (PV) against adjuvant induced arthritic rats. Arthritis was induced by intradermal injection of complete Freund's adjuvant. The activities of inflammatory, antioxidant enzymes and lipid peroxidation were estimated. PV showed high percentage of edema inhibition at a dose of 80 mg/kg on 21st day of adjuvant arthritis and is non toxic. The expression of inflammatory genes such as COX-2, iNOS, TNF-α and IL-6 and the concentration of thiobarbituric acid reactive substance were decreased by treatment with PV. Antioxidant enzymes were increased and on treatment with PV. The increased level of total WBC count and C-reactive protein in the arthritic animals was reduced in PV treated rats. Synovial cytology showed that inflammatory cells and reactive mesothelial cells were suppressed by PV. Histopathology of paw tissue showed less edema formation and cellular infiltration on supplementation with PV. Thus the results demonstrated the potential beneficial effect of PV on adjuvant induced arthritis in rats and the mechanism behind this action is due to its antioxidant and anti-inflammatory effects.
© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Arthritis is one of the most pervasive diseases that cause disability. Arthritis encompasses over 120 diseases and conditions that affect joints, surrounding tissues and connective tissues. Rheumatoid arthritis (RA) is characterized by chronic inflammation of synovial joints and subsequent progressive erosive destruction of articular cartilage [1]. The consequent morbidity and mortality have a substantial socio-economic impact and epidemiology of arthritis in female:male is 3:1 [2]. Most of the patients with aggressive disease evolution become clinically disabled within 20 years.

At present the drug used to treat RA range from non-steroidal anti-inflammatory drugs (NSAIDs) to more potent disease modifying anti-rheumatic drugs (DMARDs) [3]. The lack of reliable treatment for early RA is a major problem in modern medicine. Most of these treatments cause severe side effects such as stomach problems, heartburn, ulcers and bleeding in the case of NSAIDs and cataracts, high blood pressure, sleep problems, muscle loss, bruising, thinning of the bones (osteoporosis), weight gain and susceptibility to infections in the case of DMARDs. There is therefore a need to develop effective anti-inflammatory drugs with fewer side effects. The main objective of the present study is to evaluate the antioxidant and anti-inflammatory effect of polyphenolic fraction isolated from virgin coconut oil (VCO) on experimental arthritis.

The coconut tree is a source of various chemical compounds, which are responsible for the various bioactive properties of the tree. Recent medicinal research has confirmed many health benefits of the multiple coconut products in various forms. Hence extensive investigation is needed to exploit their therapeutic utility to combat diseases. VCO is capable of increasing antioxidant enzymes and reduces lipid peroxidation content [4]. The incredible health benefit of VCO is due to the unique type of saturated fats present in the oil. Therefore it is considered the healthiest of all dietary oils.

2. Materials and methods

2.1. Preparation of VCO

The solid endosperm of mature coconut was crushed, made into viscous slurry. The slurry was squeezed through cheese cloth to obtain

* Corresponding author. Tel.: +91 9847212268.
E-mail address: sivatheertha@gmail.com (M. Ratheesh).

http://dx.doi.org/10.1016/j.intimp.2014.02.026
1567-5769/© 2014 Elsevier B.V. All rights reserved.

coconut milk and refrigerated for 48 h. After 48 h, the milk was subjected to mild heating (50 °C) in a thermostat oven. The obtained VCO was filtered through cheesecloth and was used for the current study [5].

2.2. Isolation of polyphenolic fraction of VCO

Polyphenols from the test oils was extracted according to the method described by Vazquez Roncero et al. [6]. Ten-gram VCO was dissolved in 50-mL hexane and extracted three times with 20-mL portions of 60% methanol successively. The vacuum-dried final residue obtained from the combined extract was dissolved in a known volume of methanol. Folin–Ciocalteu reagent was used for the estimation of total polyphenol content of the solution [7].

2.3. Chemicals

All the chemicals used were high quality analytical grade reagents. Freund's complete adjuvant was purchased from Sigma Aldrich Co., USA., solvents such as chloroform, methanol, petroleum ether, ethyl acetate, hexane, ethanol, β-glycerophosphate, silica gel G, and silica gel (60–120) were purchased from Merck, India. Tissue culture plates were purchased from Tarson, India. RT-PCR kit was purchased from Eppendorf India Ltd., Chennai.

2.4. Animals

Adult male Wistar rats (weighing 150 ± 10 g) bred in the host department animal facility were used for this study. They were kept in a controlled environment for temperature (24–26 °C), humidity (55–60%) and photoperiod (12:12 h light–dark cycle). A commercial laboratory balanced diet (Amrut Laboratory Animal Feeds, Maharashtra, India) and tap water were provided ad libitum. The animals received humane care, in compliance with the host institutional animal ethics guidelines. All experiments were conducted as per the guidelines of the animal ethics committee CPCSEA (Registration No. 149/CPCSEA) according to Government of India accepted principles for laboratory animals' use and care.

2.5. Experimental design for adjuvant induced arthritis

The right hind paw of animals was immunized by injecting 0.1 mL of complete Freund's adjuvant containing heat killed mycobacteria in paraffin oil.

Animals were grouped as follows:

Group I: Normal control rats (NC)
Group II: Adjuvant induced arthritic control rats (AA)
Group III: Adjuvant induced arthritic rats supplemented with PV orally (80 mg/kg body weight in normal saline)
Group IV: Adjuvant induced arthritic rats supplemented with indomethacin orally (INDO 3 mg/kg body weight in normal saline).

Duration of experiment was 30 days. After overnight fasting, rats were sacrificed by euthanasia. For histological analysis, paw tissues were dissected, fixed in 10% buffered formalin and then decalcified for 7 days in 20% EDTA. The tissues were then processed and embedded in paraffin. Synovial fluid was obtained by injecting 100 μL of normal saline into the knee joints followed by gentle aspiration. Paw tissue and blood were also collected for various biochemical estimations.

2.6. Activity of cyclooxygenase in paw tissue

COX activity was assayed according to the method described by Shimizu et al. [8]. Tissues were incubated with Tris–HCl buffer (pH 8), 5 mM glutathione, and 5 mM hemoglobin for 1 min at 25 °C. The reaction was started by the addition of 200 μM arachidonic acid and followed by the incubation at 37 °C for 20 min. The reaction was terminated after the addition of 10% trichloroacetic acid in 1 N hydrochloric acid. Following centrifugal separation and addition of 1% thiobarbiturate, COX activity was determined by reading absorbance at 530 nm.

2.7. Superoxide dismutase activity assay

SOD activity was measured by method of Kakkar et al. [9]. Assay mixture contained 0.1 mL of supernatant, 1.2 mL of sodium pyrophosphate buffer (pH 8.3; 0.052 M), 0.1 mL of phenazine methosulphate (186 μM), 0.3 mL of nitroblue tetrazolium (300 μM) and 0.2 mL of NADH (750 μM). Reaction was initiated by addition of NADH and stopped after incubation at 30 °C for 90 s by the addition of 0.1 mL of glacial acetic acid. Following the addition of 4.0 mL of *n*-butanol and shake vigorously. Color intensity of butanol layer was measured spectrophotometrically at 560 nm. One unit of enzyme activity was defined as that amount of enzyme which caused 50% inhibition of NBT reduction/mg protein.

2.8. Catalase activity assay

CAT activity was assayed by the method of Aebi [10]. Each tissue supernatant (5 μL) was added to a cuvette containing 1.995 mL of 50 mM phosphate buffer (pH 7.0). Reaction was initiated by addition of 1.0 mL of freshly prepared 30 mM H_2O_2. The rate of H_2O_2 decomposition was measured spectrophotometrically at 240 nm.

2.9. Glutathione peroxidase assay

GPX of paw tissue was assayed in a 1 mL cuvette containing 0.890 mL of 100 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA, 1 mM NaN_3, 0.2 mM NADPH, 1 U/mL GSH reductase and 1 mM GSH. 10 μL of each paw tissue homogenate was added to make a total volume of 0.9 mL. The reaction was initiated by the addition of 100 μL of 2.5 mM H_2O_2, and the conversion of NADPH to $NADP^+$ was monitored spectrophotometrically at 340 nm for 3 min. GPX activity was expressed as nmol of NADPH oxidized to $NADP^+$/min/mg protein, using molar extinction coefficient of 6.22×10^6 (cm^{-1} M^{-1}) for NADPH [11].

2.10. Measurement of thiobarbituric acid reactive substance (TBARS)

Double heating method was used for assaying the TBARS levels in the sample [12]. 0.5 mL of each sample was mixed with 2.5 mL of trichloroacetic acid (TCA, 10%, w/v) solution and incubated in a boiling water bath for 15 min. After cooling to room temperature followed by the centrifugation at 3000 rpm for 10 min. 2 mL of supernatant from each sample was transferred to a test tube containing 1 mL of TBA solution (0.67%, w/v). The tubes were placed in a boiling water bath for 15 min. After cooling to room temperature, the purple color generated by the reaction of thiobarbituric acid (TBA) with malondialdehyde was measured spectrophotometrically at 532 nm.

2.11. Determination of NO concentrations in serum

NO was measured as its breakdown product of nitrite by using the Griess method [13]. In the presence of H_2O, NO is rapidly converted into nitrite and nitrate. Total production of NO therefore may be determined by measuring the stable NO metabolite nitrite (NO_2^-). Equal volume of paw tissue supernatant and Griess reagent (1% sulfanilamide and 0.1% N-[naphthyl]ethylenediamine dihydrochloride: 1:1) was mixed and absorbance was measured at 550 nm. The amount of nitrite was calculated from a $NaNO_2$ standard curve.

2.12. Reverse transcription-polymerase chain reaction

The gene level expression of TNF-α and IL-6 mRNA was measured by semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR). Total RNA was isolated from paw tissues using RNA isolation mini kit (Sigma Aldrich, USA) according to the manufacturer's instructions. The sequences of the primers used are shown below.

Gene	Forward primer	Reverse primer
COX-2	5′-ATCTGCCTGCTCTGGTCAATG-3′	5′-CAATCTGGCTGAGGGAACACA-3′
iNOS	5′-CAGCACAGAGGGCTCAAAGC-3′	5′-TCGTCGGCCAGCTCTTTCT-3′
GADPH	5′-GCAGGTCAGGTCCACCACTGAC-3′	5′-CGGAGTCAACGGATTTGGTCGTAT-3′
IL-6	5′-CCACTGCCTTCCCTACTTCA-3′	5′-TGGTCCTTAGCCACTCCTTC-3′
TNF-α	5′-GTCGTAAACCACCAAGC-3′	5′-ACTCCAAAGTAGACCTGCCC-3′
GAPDH	5′-CCTGCTTCACCACCTTCTTG-3′	5′-ATCCCATCACCATCTTCCAG-3′

GAPDH primers were used as an internal control for RNA loading. RT-PCR was performed in an Eppendorf thermocycler. Reverse transcription and DNA amplification was done separately with two step RT-PCR kit. For reverse transcription reaction, 2 μg of total RNA (as template), dNTPs, oligo (dT) and reverse transcriptase enzyme was used. The DNA amplification reaction included appropriate primers, PCR enzyme, cDNA formed in the first step and dNTPs. The PCR conditions were as follows: denaturation at 94 °C for 4 min; 35 cycles at 94 °C for 30 s, primer annealing for 30 s, 72 °C for 1 min; and then a final extension for 3 min at 72 °C. The PCR products were separated by electrophoresis on 1.5% agarose gel containing ethidium bromide, visualized under a UV-transilluminator and the relative intensities of bands of interest were measured on a GelDoc 2000 scanner (Bio-Rad, CA, USA) with scan analysis software.

2.13. Histopathological analysis of paw tissue

The entire paw tissue sections (5 μm) fixed by immersion at room temperature in 10% formalin solution. For histopathological examinations, paraffin-embedded paw tissue sections were stained with hematoxylin–eosin (H&E) followed by examination and photographed under a light microscope for observation of structural abnormality. The severity of paw tissue inflammation was judged by two-independent observers blinded to the experimental protocol.

2.14. Cytology of synovial fluid

Synovial fluid was obtained by injecting 100 μL of normal saline into the knee joints followed by gentle aspiration. The obtained synovial fluid was immediately fixed by ethyl alcohol:ether mixture (1:1) and stained with PAP stain.

2.15. Measurement of blood cell count and CRP

White blood cells were estimated with a hemocytometer. Plasma CRP level was measured by using DiaSys Diagnostic kit (Germany).

2.16. Assay of protein

Protein was assayed by the method of Lowry et al. [14].

2.17. Statistical analysis

Results are expressed as means ± standard error of the mean. For the results analysis, a statistical program SPSS/PC+, version 11.0 (SPSS Inc., Chicago, IL, USA) was used. One-way ANOVA was performed for comparison test of significant differences among groups. Pair fed comparisons between the groups was made by Duncan's multiple range tests. $P < 0.05$ was considered significant.

3. Results

3.1. Effect of PV on paw edema inhibition in adjuvant induced rats

PV at a dose of 80 mg/Kg bwt (Table 1) showed 74% inhibitory effect on adjuvant induced chronic model of inflammation which is higher than that of standard drug indomethacin.

3.2. Effect of PV on plasma CRP concentration

The concentration of CRP (Table 2) was increased significantly (72 ± 0.64 vs 50 ± 0.32, mg/mL, $P < 0.05$) in adjuvant induced rats when compared to normal rats. However, PV treatment significantly reduced CRP concentration (58 ± 0.44 vs 72 ± 0.64, mg/mL, $P < 0.05$).

3.3. Effect of PV on WBC count

The WBC count (Table 2) was (increased significantly 7.0 ± 0.03 vs 3.6 ± 0.02, cells × 10^3/mL, $P < 0.05$) in adjuvant induced rats when compared to normal rats. However, PV treatment significantly reduced WBC count (4. 0 ± 0.01 vs 7.0 ± 0.03, cells × 10^3/mL, $P < 0.05$).

3.4. Effect of PV on COX activity

The COX activity (Table 2) was increased significantly (8.2 ± 0.03 vs 3.9 ± 0.01, U/mg protein, $P < 0.05$) in adjuvant induced rats when compared to normal rats. However, PV treatment significantly reduced COX activity (4.3 ± 0.04 vs 8.2 ± 0.03, U/mg protein, $P < 0.05$).

3.5. Effect of PV on the activity of antioxidant enzymes

The antioxidant effect of PV on the activities of SOD, catalase, GPX was measured in paw tissue. The concentration of SOD (Fig. 1) was decreased significantly (0.5 ± 0.01 vs 4.5 ± 0.03, μmol/min, $P < 0.05$) in adjuvant induced rats when compared to normal rats. However, PV treatment significantly increases SOD concentration (5.0 ± 0.02 vs 0.5 ± 0.01, μmol/min, $P < 0.05$). The concentration of catalase (Fig. 1) was decreased significantly (2.8 ± 0.02 vs 6.3 ± 0.03, μmol/min, $P < 0.05$) in adjuvant induced rats when compared to normal rats. However, PV treatment significantly increases catalase concentration (5.9 ± 0.02 vs 2.8 ± 0.02, μmol/min, $P < 0.05$). The concentration of GPX (Fig. 1) was decreased significantly (6.2 ± 0.02 vs 12.1 ± 0.03, μmol/min, $P < 0.05$) in adjuvant induced rats when compared to normal rats. However, PV treatment significantly increases GPX concentration (12.8 ± 0.03 vs 6.2 ± 0.02, μmol/min, $P < 0.05$).

3.6. Effects of PV on the concentration of TBARS

Lipid peroxidation indicator TBARS was measured in paw tissue. The concentration of TBARS (Table 2) was increased significantly (6.9 ± 0.01 vs 2.7 ± 0.03, mmol/g, $P < 0.05$) in adjuvant induced rats when compared to normal rats. However, PV treatment significantly reduced TBARS concentration (2.98 ± 0.02 vs 6.9 ± 0.01, mmol/g, $P < 0.05$).

Table 1
The percentage inhibition of paw volume by PV.

Groups	% Inhibition of paw volume		
	8th day	14th day	21st day
Adjuvant induced	0	0	0
PV treated (80 mg/kg)	26[a]	43[a]	74[a]
Indomethacin (3 mg/kg)	26[a]	43[a]	72[a]

Values expressed as average of 6 rats in each group.
[a] Statistical difference with adjuvant induced group at $P < 0.05$.

Table 2
Inhibitory effect of PV on the concentration of plasma CRP, WBC count, TBARS, COX and nitrate.

Groups	CRP (mg/mL)	WBC (cells × 10^3/mL)	TBARS (mmol/g)	Nitrite (μmol/l)	COX (U/mg protein)
Normal	50 ± 0.32	3.6 ± 0.02	2.7 ± 0.03	9.7 ± 0.01	3.9 ± 0.01
AA	72 ± 0.64[a]	7.0 ± 0.03[a]	6.9 ± 0.01[a]	18.1 ± 0.23[a]	8.2 ± 0.03[a]
AA + PV	58 ± 0.44[a,b]	4.0 ± 0.01[a,b]	2.98 ± 0.02[a,b]	11.1 ± 0.06[a,b]	4.3 ± 0.04[a,b]
AA + IND	56 ± 0.41[a,b]	3.9 ± 0.02[a,b]	3.1 ± 0.03[a,b]	12.0 ± 0.03[a,b]	4.7 ± 0.03[a,b]

Values expressed as average of 6 samples ± SEM in each group.
[a] Statistical difference with control group at P < 0.05.
[b] Statistical difference with adjuvant rats at P < 0.05.

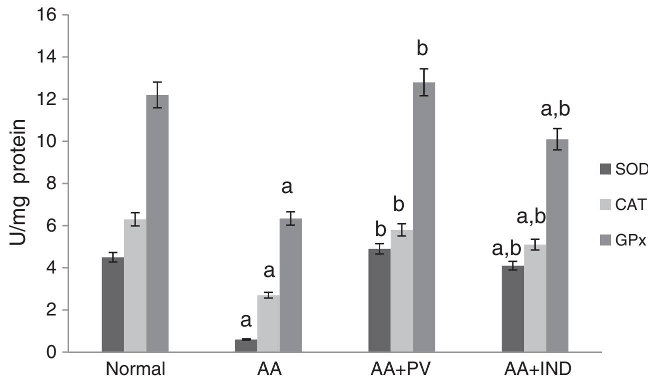


Fig. 1. Effect of PV on the activities of SOD, CAT and GPx. Values expressed as average of 6 samples ± SEM in each group. a — Statistical difference with control group at P < 0.05. b — Statistical difference with adjuvant group at P < 0.05. SOD: U-enzyme concentration required to inhibit chromogen production by 50% in 1 min. Catalase: U-μmol H_2O_2 decomposed/min. GPx: U-μmol NADPH oxidized/min.

3.7. Effect of PV on serum nitrite level

The concentration of serum nitrite (Table 2) was increased significantly (18.1 ± 0.23 vs 9.7 ± 0.01, μmol/l, P < 0.05) in adjuvant induced rats when compared to normal rats. However, PV treatment significantly reduced serum nitrite concentration (11.1 ± 0.06 vs 18.1 ± 0.23, μmol/L, P < 0.05).

3.8. Effect of PV on COX-2 and iNOS gene expression

The gene level expression of inflammatory marker genes like COX-2 and iNOS (Fig. 2A) was upregulated in arthritic rats. PV administration significantly downregulated the gene expressions on PV treated sample as compared to arthritic model.

3.9. Effect of PV on TNF-α and IL-6 gene expression

The upregulated expression of cytokines like IL-6 and TNF- α (Fig. 2 B) during arthritic induction was significantly suppressed by PV administration.

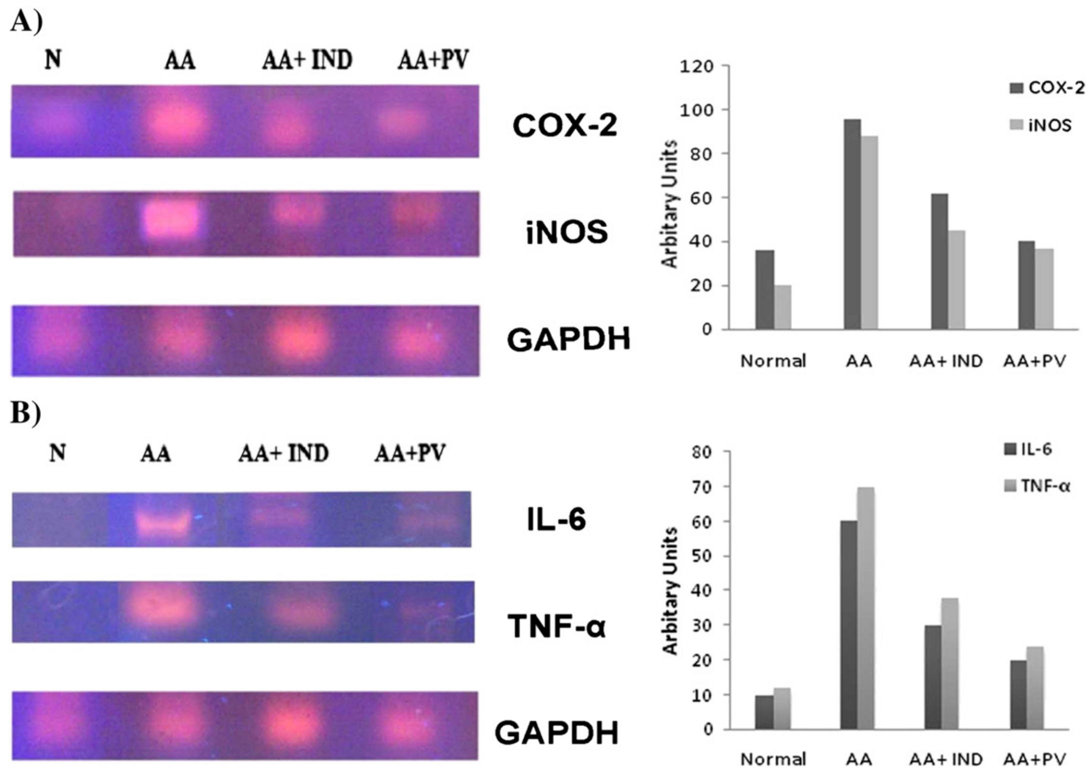


Fig. 2. (A): Inhibitory effect of PV on the expression of the proinflammatory cytokines COX-2 and iNOS. Total RNA was isolated and the expression of COX-2 and iNOS was determined by reverse transcriptase-PCR, as described in the Materials and methods section. GAPDH was used as a control. (B): Inhibitory effect of PV on the expression of the proinflammatory cytokines TNF-α and IL-6. mRNA expression by means of reverse transcriptase PCR of the mRNA isolated from paw tissue by using RNA isolation kit. For quantification, the mRNA expression data were normalized to the GAPDH signal.

3.10. Effect of PV on histopathology of paw tissue

Inflammatory cell infiltration, proliferated epithelium, proliferated collagen, and epidermal edema (Fig. 3) were markedly suppressed by treatment with PV and indomethacin as compared to adjuvant rats.

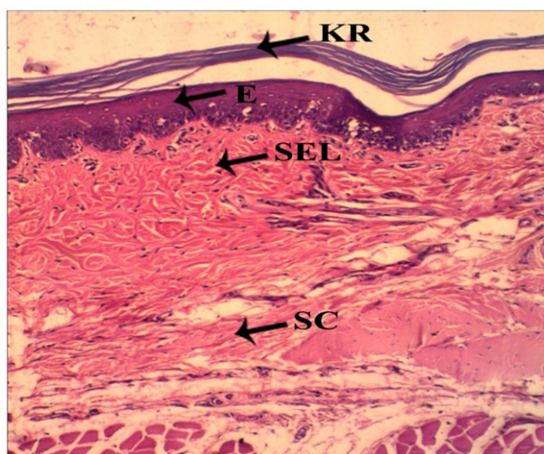
3.11. Effect of PV on cytology of synovium

Inflammatory cells and reactive mesothelial cells (Fig. 4) were suppressed by PV and indomethacin administrated rats as compared to adjuvant arthritic rats.

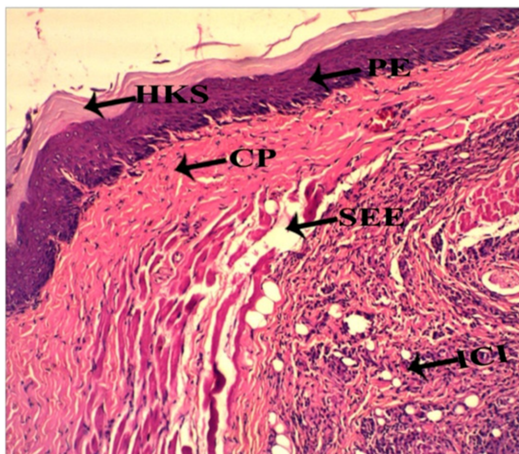
4. Discussion

Rheumatoid arthritis is a systemic chronic inflammatory disorder which may produce progressive joint damage. The release of toxic substances in the synovium during joint inflammation may lead to cartilage destruction [15]. Adjuvant induced arthritic experimental model has been used extensively for studying immuno-inflammatory processes of arthritic diseases in humans, in particular rheumatoid arthritis (RA), as well as for screening and testing novel anti-arthritic agents [16]. The therapeutic efficacy of the drugs and degree of inflammation was determined by observing the paw swelling of experimental rats. The edema formation in peri-articular tissues such as ligament and joint capsule leads to soft tissue swelling around ankle joints in arthritic rats. In the present study, chronic inflammation was induced by Freund's complete adjuvant in rats that developed a chronic swelling in multiple joints, with influence of inflammatory cells, erosion of joint cartilage and bone destruction. The release of number of inflammatory mediators during chronic inflammation leads to pain, destruction of bone and cartilage that can lead to severe disability. In the present study, administration of 80 mg/kg of PV showed significant inhibition of paw edema volume and the inhibition was higher than that of indomethacin treated group.

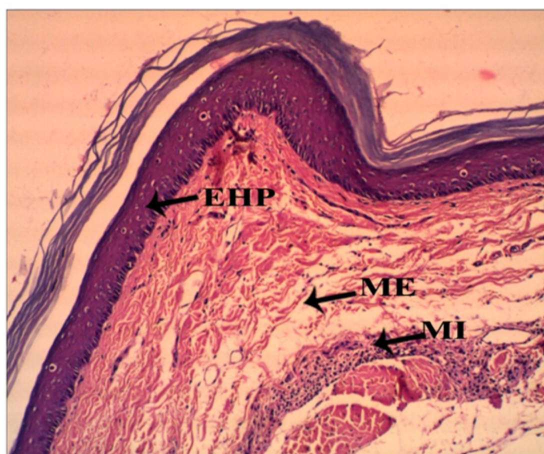
In our study the level of CRP and total WBC count were increased. Increased CRP in adjuvant induced rats which show the release of acute phase protein into the blood in first phase of adjuvant induction and administration of PV decreased the CRP in plasma and the level was lower than that of indomethacin treated rats, which support the present inhibition of paw swelling in PV treated rats.



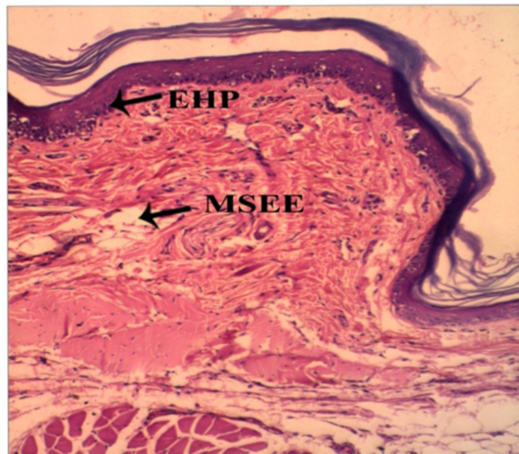
A) ***Histopathology of paw tissue of normal rat***
Cross section of normal paw tissue shows keratin(KR), Epidermis(E), Sub epidermal layer(SEL),Sub cutaneous layer (SC).



B) ***Histopathology of paw tissue of AA rat***
Cross section of adjuvant induced arthritis paw tissue shows Massive influx of inflammatory cell infiltration (ICI).Proliferated collagen(CP),Proliferated epithelium (PF),Hyper keratotic skin(HKS),Sub epidermal edema (SEE)

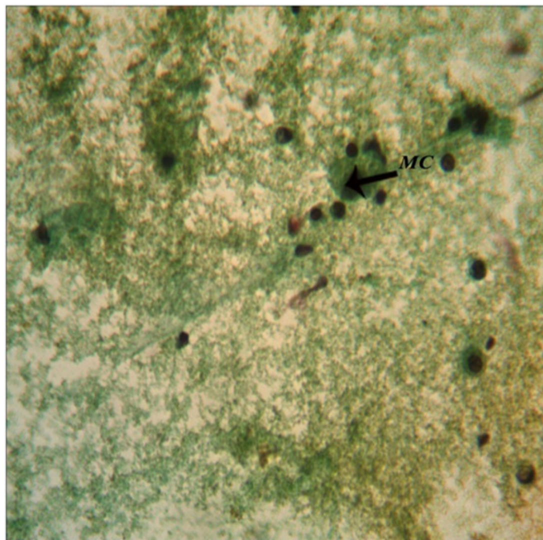


C) ***Histopathology of paw tissue of AA + IND rat***
Cross section of the paw tissue shows Mild edema(ME),Mild inflammation(MI),Mild epithelial hyper plasia **(EHP).**

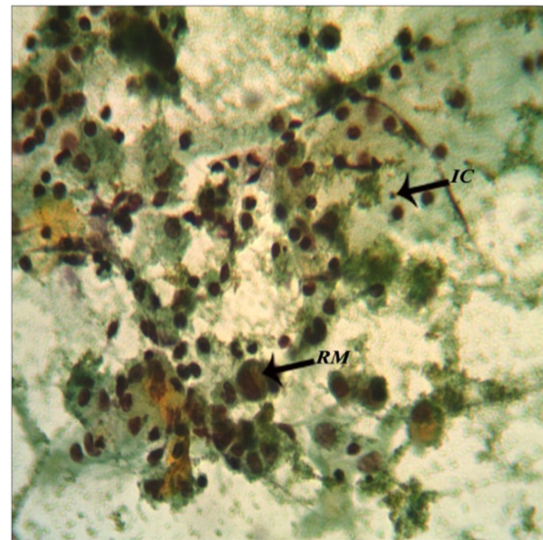


D) ***Histopathology of paw tissue of AA+PV rat***
Cross section of tissue shows Mild sub epidermal edema (MSEE),Mild epithelial hyper plasia(EHP).

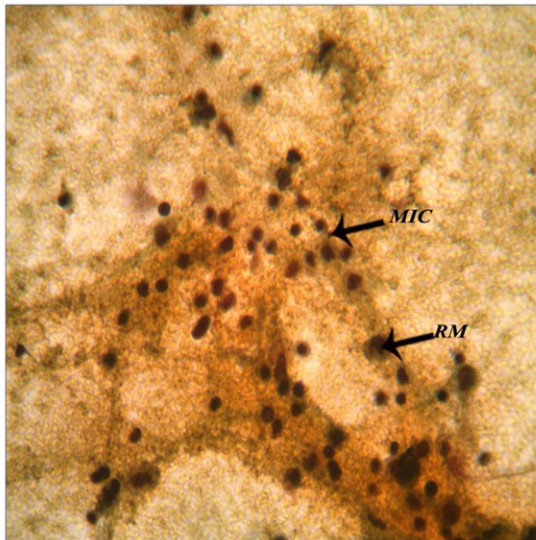
Fig. 3. Histology of paw tissue (H&E stain 40×). Values expressed as average of 3 samples ± SEM in each group. AA treated group showed inflammatory cell infiltration, proliferated epithelium, proliferated collagen and epidermal edema as compared to normal group. AA + PV treated group showed mild edema, mild inflammation and mild epithelial hyperplasia as compared to AA treated group.



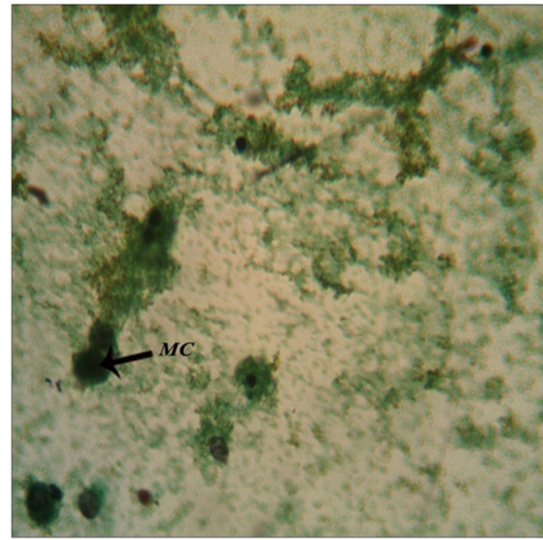
A) *Cytology of synovium from normal rat*
cytology of synovium from normal rat shows Mesothelial cells(MC).



B) *Cytology of synovium from AA rat*
Cytology of AA rat shows Inflammatory cells(IC),Reactive mesothelial cells(RM).



C) *Cytology of synovium from AA + IND rat*
Cytology of AA + IND rat shows mild Inflammatory cells (MIC),Reactive mesothelial cells(RM).



D) *Cytology of synovium from AA + PV rat*
Cytology of AA+ PV rat shows Mesothelial cells(MC).

Fig. 4. Cytology of synovium from rat. Values expressed as average of 3 samples ± SEM in each group. Inflammatory cells and reactive mesothelial cells were seen in AA treated group as compared to normal group. AA + PV treated group shows only mesothelial cells as compared to AA.

IL-6 is reported to play devastating role in cartilage and bone degradation during arthritis. IL-6 unregulated production of cytokines like IL-8, matrix metalloproteinases and adhesion molecules by inflammatory cells in the synovium [17]. IL-6 is also reported to stimulate secretion of collagenase and PGE_2 by synovial cells contributing to joint damage in inflammatory conditions like rheumatoid arthritis [18]. In the present study APPs were found elevated in arthritic rats. In the present study, PV supplementation significantly down regulated the expression of IL-6 in paw tissue of arthritic rats. PV supplementation also restored the levels of APPs to near normal values in arthritic rats. Thus decreased IL-6 production correlated with decreased APP production thereby reducing disease severity.

The evaluation of paw swelling is a simple and quick procedure for evaluating the degree of inflammation and the therapeutic efficacy of the drugs. Arthritic rats showed the soft tissue swelling around ankle joints that was considered to be due to edema of peri-articular tissues such as ligament and joint capsule. Prostaglandins induce the relaxation of arteriolar smooth muscle cells and increase the blood supply to the tissue. The ability of the drug to reduce edema formation may thus be related to its inhibitory action on prostaglandin synthesis. COX-1 and COX-2 are two distinct but related enzymes, which is responsible for prostaglandin synthesis. The COX-1 is a constitutive enzyme, responsible for maintaining normal renal function, gastric mucosa integrity and hemostasis [19]. During inflammatory conditions, the inducible isoform COX-2 enhances the production of prostaglandins that are associated with inflammation. The present study shows that administration of PV significantly reduces paw edema volume in arthritic rats. Also the activity of total COX and COX-2 expressions was decreased in PV treated groups. Reduction of paw swelling and decreased expression of COX-2 indicate the immunological protection provided by the PV.

Increased levels of TNF-α were found in sera and synovial fluid of RA patients, suggesting that TNF-α may play a role in the pathology of the

disease. Blocking of TNF-α followed by inactivation of T cells, macrophages, and fibroblasts interrupts the inflammatory process [20]. Anti-TNF-α therapy and cytokine blockers reduce the abnormalities including morning stiffness, pain score and swollen joint count [21]. On pharmacological basis, the anti-TNF-α activity supports the usage of PV as a medicinal herb for the treatment of rheumatoid arthritis and related inflammatory disorder.

Production of NO in suitable magnitude serves as a key-signaling molecule in various physiological processes. But at the same time NO has been found to cause pathological conditions in chronic inflammation [22]. Our study showed that nitrite production in PV treated animals was significantly lower as compared to adjuvant treated rat. This suggests that reduction of chronic inflammation in arthritic animals might be due to the downregulation of iNOS expression followed by decreased cellular production of NO. Therefore, the inhibitory activity of PV may be due to their anti-inflammatory properties and ability to counteract NO induced oxidative damage, which eventually helps in remodeling of cells.

The superoxide radicals are the first product of molecular oxygen reduction. In vivo protection of cells and tissues from superoxide radicals and other peroxides such as lipid peroxides was performed by SOD. It acts as a catalyst for dismutation reactions through the conversion of superoxide radicals into H_2O_2 and molecular oxygen [23]. The first line of defense against peroxidation was provided by glutathione, which endogenously synthesized in the liver [24]. The decrease in glutathione contributes to increased cellular damage by favoring attack by free radicals. Polyphenols from virgin coconut oil enhances the activity of GSH and SOD and preserve the integrity of cellular membranes.

Glutathione peroxidase (GPX) and catalase activity were markedly reduced in arthritic condition. The accumulation of H_2O_2 which in turn causes the inhibition of GPX and catalase enzymes leads to the accumulation of highly reactive lipid hydroperoxide in the aqueous phase of cell membranes [25]. Selenium level in tissue had a close relation with GPX activity. Selenium deficiency results in pathological condition. In rheumatoid arthritis, the lower selenium level was reported which could account for the decreased activity of GPX [26]. PV administration produced a significant increase in free radical scavenging activity of antioxidant enzymes like GPX and catalase.

Lipid peroxidation is one of the critical mechanisms of injury that associated with RA [27]. Tissue thiobarbituric acid reactive substance (TBARS) analysis is an extensively used method for evaluating lipid peroxidation in tissue. In the present investigation, the increased levels of TBARS were observed in arthritic rats. The elevated levels of TBARS were significantly decreased after the treatment of PV. PV protects against free radicals formation and reduces the inflammation. These observations suggested that adjuvant induced arthritis may be associated with lipid peroxidation and anti-arthritic effect of PV reduce lipid peroxidation and causing a modulation in cellular antioxidant defense system.

Histopathological studies indicated that inflammatory cell infiltration, proliferated epithelium, proliferated collagen, and epidermal edema were markedly suppressed in PV administrated rats. Synovial cytology showed that inflammatory cells and reactive mesothelial cells were suppressed by PV and indomethacin administrated rats as compared to adjuvant arthritic rats.

Thus, present study showed that the polyphenolic fraction of virgin coconut oil markedly reduces cell influx; release of mediators, lipid peroxidation and oxidative stress associated with arthritic condition, and therefore has the potential to be used as an anti-arthritic agent.

Acknowledgment

We express our sincere thanks to Shri. T. K. Jose IAS, Honorable Chairman, Coconut Development Board, Cochin and, Dr. K. Muralidharan, Director, CDB, for their immense support and valuable suggestions.

References

[1] Feldmann M, Brennan FM, Maini RN. Role of cytokines in rheumatoid arthritis. Annu Rev Immunol 1996;14:397–440.
[2] Narendirakannan RT, Subramaniam S, Kandaswamy M. Anti-inflammatory and lysosomal stability actions of *Cleome gynandra* L. studied in adjuvant induced arthritic rats. Food Chem Toxicol 2007;45:1001–12.
[3] Rathore Brijesh, Ali Mahdi Abbas, Nath Paul Bhola, Narayan Saxena Prabhu, Kumar Das Siddharth. Indian herbal medicines: possible potent therapeutic agents for rheumatoid arthritis. J Clin Biochem Nutr 2007;41(1):12–7.
[4] Nevin KG, Rajamohan T. Beneficial effects of virgin coconut oil on lipid parameters and in vitro LDL oxidation. Clin Biochem 2004;37(9):830–5.
[5] Nevin KG, Rajamohan T. Virgin coconut oil supplement diet increases the antioxidant status in rats. Food Chem 2006;99:260–6.
[6] Vazquez Roncero A, Janer del valle L, Janer del valle C. Determination of olive oil total polyphenols. GrassaAceites 1973;24:350–5.
[7] Gutfinge T. Polyphenols in olive oil. J Am Oil Chem Soc 1981;1:966–8.
[8] Shimizu T, Kondo K, Hayaishi O. Role of prostaglandin endoperoxides in the serum thiobarbituric acid reaction. Arch Biochem Biophys 1981;206:271–6.
[9] Kakkar P, Das B, Viswanathan P. A modified spectrophotometric assay of superoxide dismutase. Ind J Biochem Biophys 1984;21:130–2.
[10] Aebi H. Catalase in vitro. Methods Enzymol 1984;105:121–6.
[11] Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967;70:158–69.
[12] Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol 1990;186:421–31.
[13] Grisham MB, Johnson GG, Lancaster JR. Quantitation of nitrate and nitrite in extracellular fluids. Methods Enzymol 1996;268:237–46.
[14] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193(1):265–75.
[15] Darlington LG, Stone TW. Antioxidants and fatty acids in the amelioration of rheumatoid arthritis and related disorders. Br J Nutr 2001;85:251–69.
[16] Billingham ME. Models of arthritis and the search for antiarthritic drugs. Pharmacol Ther 1983;21:389–428.
[17] Thalhamer T, McGrath MA, Harnett MM. MAPKs and their relevance to arthritis and inflammation. Rheumatology 2008;47:409–14.
[18] Anderson GD, Hauser SD, McGarity KL, Bremer ME, Isakson P, Gregory SA. Selective inhibition of cyclooxygenase-2 and interleukin-6 in rat adjuvant arthritis. J Clin Invest 1996;97:2672–9.
[19] Griswold DE, Adams JL. Constitutive cyclooxygenase (COX-1) and inducible cyclooxygenase (COX-2): rationale for selective inhibition and progress to date. Med Res Rev 1996;16:181–6.
[20] Yazdani-Biuki B, Stadlmaier E, Mulabecirovic A, Brezinschek R, Tilz G, Demel U. Blockade of tumor necrosis factor α significantly alters the serum levels of IgG- and IgA-rheumatoid factor in patients with rheumatoid arthritis. Ann Rheum Dis 2005;64:1224–6.
[21] Camussi G, Lupia E. The future of antitumour necrosis factor products in the treatment of rheumatoid arthritis. Drugs 1998;55:613–62.
[22] Gross SS, Wolin MS. Nitric oxide: pathophysiological mechanisms. Annu Rev Physiol 1995;57:737–69.
[23] Okabe T, Hamaguchi K, Inafuku T, Hara M. Aging and superoxide dismutase activity on cerebrospinal fluid. J Neurol Sci 1996;141:100–4.
[24] Walz DT, Dimartino MJ, Misher A. Adjuvant-induced arthritis in rats. II. Drug effects on physiologic, biochemical, and immunologic parameters. J Pharmacol Exp Ther 1971;178:223–31.
[25] Hassan MQ, Hadi RA, Al-Rawi ZS, Padron VA, Stohs SJ. The glutathione defense system in the pathogenesis of rheumatoid arthritis. J Appl Toxicol 2001;21:69–73.
[26] Parnham MJ, Graf E, Hoff E, Niemann R. Selenium hydroperoxides and atherosclerosis. Agents Action 1987;22:3–4.
[27] Gambhir JK, Lali P, Jain AK. Correlation between blood antioxidant levels and lipid peroxidation in rheumatoid arthritis. Clin Biochem 1997;30:351–5.