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

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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 beneficiary effect of PV on adjuvant induced arthritis in rats and the mechanism behind this action is due to its antioxidant and anti-inflammatory effects.
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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

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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.

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