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Research Article
Effect of *Trichilia monadelpha* (Thonn.) J. J. De Wilde (Meliaceae) Extracts on Haematology, Cytokines and Oxidative Stress Biomarkers in Rats Adjuvant-Induced Arthritis

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Abstract

Background and Objectives: Cytokines and oxidative stress biomarkers are elevated in arthritis. This study therefore investigated the effects of petroleum ether, ethanol and ethyl acetate extracts of *Trichilia monadelpha* stem bark on haematological profile, serum cytokine levels and tissue oxidative stress biomarkers in arthritic rats. **Methodology:** Hematological profile, assay of interleukin-6 and tumour necrosis factor-α, tissue proteins as well as malondialdehyde, myeloperoxidase and superoxide dismutase in arthritic tissue was carried out in groups of CFA-induced (sub-plantar injection of 0.1 mL of 5 mg mL^{-1} of heat killed *Mycobacterium tuberculosis* in paraffin oil) arthritic Sprague-Dawley rats treated with either 10, 30 and 100 mg kg^{-1} of the extracts, 0.3-3.0 mg kg^{-1} dexamethasone or 0.1-1.0 mg kg^{-1} methotrexate over a 28 day period. **Results:** The extracts restored ($p<0.01$-0.0001) the significantly elevated WBCs, neutrophils and lymphocytes and the significantly reduced RBC and haemoglobin, associated with arthritis, to normal. The extracts ($p<0.01$-0.001), dexamethasone ($p<0.05$-0.01) and methotrexate ($p<0.05$-0.01) significantly and dose-dependently reduced high levels of TNF-α and IL-6 as well as malondialdehyde and myeloperoxidase associated with arthritis, relative to the control. Superoxide dismutase activity also increased significantly ($p<0.01$-0.0001) with treatments. **Conclusion:** The petroleum ether, ethyl acetate and ethanol extracts of *Trichilia monadelpha* stem bark improved haematological features, decreased tissue proteins, cytokines and oxidative stress biomarkers indicating its potent anti-inflammatory and antioxidant property and establishing its usefulness in the traditional management of arthritis.

Key words: Superoxide dismutase, malondialdehyde, myeloperoxidase, quantikine rat immunoassay, *Trichilia monadelpha*

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Rheumatoid arthritis is a long-term inflammatory disease of joints that causes pain, swelling and stiffness[1]. This feature is associated with a systemic increase in pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α)[2] and oxidative stress[3]. These cytokines are found in higher concentrations in synovial fluid and represent key mediators in the pathogenesis of RA[1]. Synovial inflammation in RA spreads systemically and transforms into chronic inflammation showed by increased cytokine release and Reactive Oxygen Species (ROS)[3].

Effective management of RA would therefore require potent anti-inflammatory agent (orthodox or alternative). Rheumatoid arthritis is a long-term inflammatory disease of joints that causes pain, swelling and stiffness[1]. This feature is associated with a systemic increase in pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α)[2] and oxidative stress[3]. These cytokines are found in higher concentrations in synovial fluid and represent key mediators in the pathogenesis of RA[1]. Synovial inflammation in RA spreads systemically and transforms into chronic inflammation showed by increased cytokine release and Reactive Oxygen Species (ROS)[3].

Effective management of RA would therefore require potent anti-inflammatory agent (orthodox or alternative). Drugs that work via several mechanisms of anti-inflammation would be of immense therapeutic advantage. Cytokine-targeted drugs, NSAIDs, DMARDs and corticosteroids are often used to manage RA[4,5]. Although, studies have indicated that TNF-α blockers did not further increase this risk in patients with RA[6], it may enhance cancer risk, in particular the risk of developing lymphoma in children and in adolescents[7]. The targeting of IL-6 holds therapeutic potential[8] however a reduction in its levels could make an individual highly susceptible to infection as IL-6 is a central mediator of the immune system inducing; the liver acute-phase response and optimal B cell and T cell effector responses to pathogens. Thus, the potential beneficial effects of long-term IL-6 neutralization for patients suffering from chronic inflammatory diseases may be outweighed in some cases by adverse effects[7].

The NSAIDs may cause gastrointestinal disorders (such as gastric and duodenal ulcers, small intestinal perforation and strictures and colitis) and bleeding disorders[9]. It could cause renal disorders by reduce renal blood flow and glomerular filtration rate as well as some allergic disorders[10]. The DMARDs can cause skin, liver, kidney and gastrointestinal side effects[11] while the corticosteroids could causes cardiovascular system effects like dyslipidaemia and hypertension[12], The most common adverse effects of short-term corticosteroid therapy are euphoria and hypomania, meanwhile, long-term therapy tends to induce depressive symptoms.

The search for natural products that have potent anti-inflammatory effect in RA with lesser side-affects and are affordable to the individuals, who do not have easy accessibility to orthodox medicines, living in developing countries are being carried out. One of such is, *Trichilia monadelpha* (*Meliaceae*), a plant reported to have anti-inflammatory[13] and analgesic properties[13,14]. It improves sperm viability[15] is relatively safe to use[13] and contains important secondary metabolites that improves health[13,14,16].

This study therefore sought to determine the effect of a petroleum ether, ethyl acetate and an ethanolic extracts of *Trichilia monadelpha* stem bark on the haematological profile, serum cytokines levels and tissue oxidative stress biomarkers of arthritis in Sprague-Dawley rats.

MATERIALS AND METHODS

Experimental animals: Male Sprague-Dawley rats (150-200 g) obtained from Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, were kept in the animal house of the Department of Pharmacology, KNUST, Kumasi, Ghana. Animals were housed in aluminium cages and fed with normal rat diet (GHAFCO, Tema, Ghana) and water, *ad libitum*. Rats were kept according to the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH, Department of Health and Human Services publication no. 85-23, revised 1985), approved by the Departmental Ethics Committee.

Drugs and chemicals used: Heat killed *Mycobacterium tuberculosis*, strains C, DT and PN, mixed (Ministry of Agriculture, Fisheries and Food, U.K) was used to induce arthritis. All chemicals used for the antioxidant assay were bought from Sigma-Aldrich, Inc. St. Louis MO USA. Quantikine rat IL-6 and TNF-α Immunoassay kit R and D Systems, Inc., Minneapolis, USA) were used to assay IL-6 and TNF-α levels. Dexamethasone (Wuhan Grand, China) and methotrexate (Dabur Pharma, India) were the reference drugs used for treatment of arthritis.

Plant extraction and dosing: Extraction and labelling of the petroleum ether, ethyl acetate and ethanol extracts of stem bark of *Trichilia monadelpha* as PEE, EAE and EthE

respectively, was as described by Ben *et al*[16]. The extracts, were triturated with Tween-80 (3 drops) in Normal Saline (NS) and administered orally to rats at doses ranging from 10-100 mg kg^{-1}.

Haematology: Blood samples obtained from arthritic rats, by cardiac puncture were put into K3 EDTA sterilized sample tubes (VACUETTE®, USA). Haemoglobin concentration (Hb), Red Blood Cell (RBC), total White Blood Cell (WBC), lymphocytes, neutrophils and platelet counts and Haematocrit or Packed Cell Volume (PCV) were determined using the KX-21 N Automated Hematology Analyzer (Sysmex Corporation, Chuo-ku, Kobe, Japan).

Assay of oxidative stress biomarkers: At the end of the experimental period paws of rats were harvested, rinsed, weighed and put into sample tubes each containing 5 mL of 0.01 M Phosphate Buffered Saline (PBS) per 1 g of paw weight. The paws were then freeze-thawed in liquid nitrogen and homogenised for 10 min using Ultra-Turrax T25 homogeniser (Janke and Kunkel GmbH and Co. KG. IKA Labortechnik, Staufen, Germany). The homogenates were centrifuged (temperature: 25°C, speed: 4000 g) for 5 min using the Mikro 220R [HettichZentrifuge, USA] to obtain supernatant solutions for assay of protein and oxidative stress biomarkers.

Protein assay: The protein concentration of the supernatant solutions obtained was determined using the modified Lowry's protein assay method[17,18]. Briefly, dilute samples (0.025-0.25 mg mL^{-1}) with buffer (Sodium carbonate (20 g) dissolved in 260 mL water, 0.4 g cupric sulphate (5×hydrated) in 20 mL water and 0.2 g sodium potassium tartrate in 20 mL water. All three solutions were mixed to prepare the copper reagent) was prepared. Each dilution was prepared at 400 µL. Samples were prepared in duplicates. A blank of 400 µL buffer was prepared. Standards from 0.25 mg mL^{-1} Bovine Serum Albumin (BSA) was prepared by adding 40-400 µL of BSA to 13×100 mm tubes and made up to 400 µL buffer solution/tube 400 µL of 2×Lowry concentrate (3 parts copper reagent mixed with 1 part SDS (100 mL of 1% solution (1 g/100 mL) of sodium dodecyl sulphate, SDS) and 1 part NaOH, (1 M solution of NaOH (4 g/100 mL)) was mix thoroughly and incubated at room temperature 10 min. After incubation, 200 µL 0.2 N Folin reagent was added and vortexed immediately. This was incubated at room temperature for 30 min. Absorbance was taken at 700 nm using UV mini-1240 single beam spectrophotometer (Shimadzu Scientific Instruments (SSI), Kyoto, Japan). A linear curve of standard, BSA, was obtained by plotting various concentrations versus absorbance. The protein equivalent (mg) in tissue, extrapolated from standard (BSA) was used in calculations of the biochemical markers of oxidative stress evaluated.

Tissue malondialdehyde levels: Malondialdehyde (MDA) was measured according to procedure described by Reilly[19]. A 1×working solution of TBA/TCA/HCl reagent was prepared by diluting the stock solution 4-fold in water. While stirring the solution with a magnetic stir bar, BHT was added to a final concentration of 0.03%. The supernatant and blank (without sample) was combined with the TBA/TCA/HCl reagent at a reagent/sample ratio of 2:1 (v/v). This was mixed thoroughly and placed in a boiling water bath for 15 min. The mixture was allowed to cool to room temperature and then centrifuged at 1000×g for 10 min at room temperature. The absorbance of solution was read at 535 nm against the blank using UV mini-1240 single beam spectrophotometer (Shimadzu Scientific Instruments (SSI), Kyoto, Japan). Concentration of MDA was calculated as specific activity (U mg^{-1}) using the extinction coefficient of 1.56×105 M^{-1} cm^{-1} [20] as follows:

$$\text{Volume activity } (\text{U mL}^{-1}) = \frac{\Delta A_{535}}{V_S \times \varepsilon} \times \text{dilution factor} - 1$$

$$\text{Weight activity } (\text{U mg}^{-1}) = \frac{\text{Volume activity } (\text{U mL}^{-1})}{\text{Material (tissue)} (\text{mg mL}^{-1})}$$

$$\text{Specific activity } (\text{U mg}^{-1}) = \frac{\text{Weight activity } (\text{U mg}^{-1})}{\text{Protein (mg)}}$$

Tissue myeloperoxidase levels: The assay and units of myeloperoxidase (MPO) activity were performed and calculated as described by Ormrod *et al*[21]. Separated sediments from centrifugation of homogenized paws were suspended in 2 mL 0.05 M PBS (pH 6.0) containing 0.5% cetrimide. The suspension was freeze-thawed three times and finally centrifuged at 1000 g for 10 min at room temperature. A 1 cm path length cuvette, contained 873 µL of 0.05 M PBS (pH 6.0), 30 µL of undiluted supernatant, 100 µL of o-dianisidine dihydrochloride[22] and 10 µL aminotriazole (AMT). This was allowed to incubate at room temperature for 3 min so AMT could inhibit eosinophil peroxidase (EPO) present. Finally 10 µL of 0.5 mM H_2O_2 was added to the cuvette and change in absorbance was read at 460 nm every 5 sec for 3 min. MPO was expressed as units where 1 unit is defined as

that degrading 1 µmol of peroxide/min at 25°C using the absorbance index of H_2O_2 of 11.3 (µmol $mL^{-1})^{-1}$ cm^{-1}. Calculated as follows:

$$\text{MPO total OD } (\text{sec}^{-1}) = (\Delta\text{MPO OD sec}^{-1}) \times \frac{1+\text{weight of sample}}{0.03}$$

$$\text{MPO total units} = \frac{\text{MPO total OD } (\text{sec}^{-1})}{11.3}$$

$$\text{MPO of protein (mg)} = \frac{\text{MPO total units}}{\text{Protein (mg)}}$$

Tissue total superoxide dismutase activity: The total superoxide dismutase[23] activity was measured using xanthine oxidase/xanthine/cytochrome c method[24]. This method involves competition for xanthine and xanthine oxidase generated superoxide radical between SOD and cytochrome c. Action of SOD produces a colour complex that was measured at 550 nm for 5 min at 1 min interval to obtain ΔA550 nm min^{-1}. SOD reaction cocktail and xanthine oxidase (XOD) assay medium consisted of a reaction mixture of 2.8 mL of SOD reaction cocktail, 0.1 mL XOD solution and 0.1 mL sample in a 3 mL cuvette of 1 cm path length. The blank consisted of 2.8 mL of SOD reaction cocktail, 0.1 mL XOD solution and 0.1 mL distilled water in a 3 mL cuvette of 1 cm path length. SOD activity was expressed as U/mg protein. A unit is that which inhibits the rate of reduction of cytochrome c by 50% in a coupled system using xanthine and xanthine oxidase at pH 7.8 at 25°C in a 3 mL reaction volume. The SOD activity was calculated as follows:

$$\text{Inhibition (\%)} = \frac{\Delta A_{550}\ \text{test} - \Delta A_{550}\ \text{control}}{\Delta A_{550}\ \text{control} - \Delta A_{550}\ \text{blank}} \times 100$$

$$\text{Volume activity (U mL}^{-1}\text{ enzyme)} = \frac{\text{Inhibition (\%)} \times \text{df}}{50\% \times 0.1}$$

$$\text{Specific activity (U mg}^{-1}\text{ protein)} = \frac{\text{Enzyme (U mL}^{-1})}{\text{Protein (mg)/ Enzyme (mL)}}$$

where, df is dilution factor of sample, 50% is inhibition of the rate of cytochrome c reduction as per the unit definition 0.1-Volume[25] of enzyme (XOD) used.

Serum tumour necrosis factor-α and interleukin-6 levels: Serum TNF-α and IL-6 was analysed using quantitative sandwich enzyme immunoassay technique. Blood obtained by cardiac puncture from various treatment groups was allowed to clot at room temperature in test tubes and centrifuged at 1000×g for 20 min. Serum obtained was assayed for TNF-α and IL-6 described in the kit manual.

A monoclonal antibody specific for rat TNF-α and IL-6 had been precoated on to a microplate. Standards control and samples were pipetted into the wells and any rat TNF-α and IL-6 present is bound by the immobilised antibody. The wells were washed to remove any unbound substances. After washing an enzyme-linked polyclonal antibody specific for these cytokines were added into the wells. A substrate solution was added to the wells after washing to remove any unbound antibody-enzyme reagent. The enzyme reaction yielded a blue product that turned yellow when a Stop solution was added. The intensity of the colour was measured at 450 nm using Sunrise microplate reader (XREAD PLUS version: V4.30, Tecan Inc., Switzerland) powered by Smart Magellan data analysis software. Absorbance is equivalent to the amount of cytokines in standard. The sample values were read off the standard curve.

Statistical analysis: Significant differences in haematological values, oxidative stress biomarkers and cytokines levels were analysed by one-way analysis of variance (1-way ANOVA) and Holm-Sidak's *post hoc* test using SigmaPlot version 12.3 (Systat Software Inc. Chicago USA). All values were expressed as Mean±SEM. $p \leq 0.05$ and higher F values ($F \geq 4.0$) were considered significant.

RESULTS

Haematology: There were significant decrement ($p<0.0001$) in Hb, RBC and PCV in arthritic rats. These decrements were reversed to within normal range with treatments by PEE, EthE, EAE, dexamethasone and methotrexate. White blood cells and platelets were however significantly ($p<0.05$) reduced by extracts and reference drugs treatments (Table 1).

Tissue malondialdehyde (MDA): Malondialdehyde MDA levels were significantly ($p<0.0001$) high in the CFA group however; PEE, EthE and EAE-treated groups showed a significant reduction (ipsilateral paw: $F_{4,10} = 473.30$ $p<0.0001$; $F_{4,10} = 2779.00$ $p<0.0001$; $F_{4,10} = 5649.00$ $p<0.0001$, respectively; contralateral paw: $F_{4,10} = 1169.00$ $p<0.0001$; $F_{4,10} = 1642.00$ $p<0.0001$; $F_{4,10} = 1478.00$ $p<0.0001$, respectively) in MDA levels. The maximal effect (97.8% at 30 mg kg^{-1}, 99.7% at 10 mg kg^{-1}, 97.9% at 30 mg kg^{-1},

Table 1: Haematological parameters profile of *Trichilia monadelpha* extracts and reference drugs in normal and experimental rats

Parameters	WBC (µL)	RBC (µL)	Hb (g dL^{-1})	PCV (%)	PLT (µL)	LYM (%)	NEUT (%)
IFA	5.40±1.10×10^3	7.77±0.21×10^6	13.40±0.00	45.55±0.95	778.0±13.00×10^3	31.75±3.15	27.55±3.65
CFA	19.10±1.70×10^3	5.00±0.10×10^6	6.55±0.35	24.10±3.50	3070±499.5×10^3	89.55±8.95	70.85±7.45
PEE (mg kg^{-1})							
10	13.20±1.30×10^3	8.55±0.71×10^{6***}	14.20±0.20***	49.90±1.90**	681.5±116.5×10^{3***}	60.15±11.75	41.90±11.20
30	12.80±2.10×10^3	7.50±0.16×10^{6**}	11.90±0.50***	42.10±1.40	949.0±267.0×10^{3***}	57.20±9.70	38.45±9.75
100	14.25±0.95×10^3	8.00±0.11×10^{6**}	12.90±0.20***	45.05±0.85*	737.0±29.0×10^{3***}	71.60±5.40	29.35±3.75**
EthE (mg kg^{-1})							
10	10.25±2.05×10^3	7.92±0.17×10^{6**}	13.05±0.75***	46.15±1.75*	683.0±244.0×10^{3***}	69.50±1.60	53.95±2.75
30	10.55±2.25×10^3	7.50±0.28×10^{6**}	12.90±0.30***	44.50±01.60*	789.0±192.0×10^{3***}	68.50±6.10	57.75±0.95
100	9.65±3.85×10^3	7.80±0.07×10^{6**}	12.70±0.50***	43.60±1.70*	767.5±87.5×10^{3***}	77.25±11.25	51.50±2.80
EAE (mg kg^{-1})							
10	12.85±1.45×10^3	7.89±0.27×10^{6**}	14.90±0.40***	47.15±1.95**	794.5±24.50×10^{3***}	52.60±3.80	58.15±4.95
30	8.90±1.30×10^3	8.74±0.48×10^{6***}	15.95±0.35***	50.20±1.50**	601.5±57.50×10^{3***}	42.60±8.60	55.20±5.10
100	9.35±1.95×10^3	7.63±0.03×10^{6**}	14.60±0.10***	46.20±0.90*	567.5±206.5×10^{3***}	56.25±13.05	59.05±0.95
DEX (mg kg^{-1})							
0.3	9.70±4.70×10^3	8.01±0.33×10^{6**}	14.55±0.05***	50.65±0.25**	787.5±71.50×10^{3***}	44.70±10.40	57.40±8.6
1.0	5.00±0.00×10^{3*}	8.64±0.07×10^{6***}	14.75±0.35***	49.85±3.25**	730.5±56.50×10^{3***}	35.55±3.45*	34.85±1.15*
3.0	4.65±0.35×10^{3*}	8.80±0.20×10^{6***}	15.50±0.70***	57.70±1.00***	720.5±266.50×10^{3***}	33.30±0.50*	30.10±0.50**
MET (mg kg^{-1})							
0.1	8.45±0.25×10^3	8.45±0.25×10^{6***}	12.35±0.55***	43.05±1.85	937.5±482.5×10^{3***}	62.15±11.85	50.85±5.95
0.3	5.15±0.15×10^{3*}	9.20±0.30×10^{6***}	14.80±0.70***	57.20±9.70***	625.0±95.0×10^{3***}	38.15±3.65*	37.15±3.55
1.0	5.25±1.15×10^{3*}	9.60±0.80×10^{6***}	13.05±0.65***	51.05±4.55**	758.5±227.5×10^{3***}	31.75±1.45**	31.95±2.15*

***p<0.001, **p<0.01, *p<0.05 compared to Arthritic group[54], WBC: White blood cells, RBC: Red blood cells, PHB: Heamoglobin, PCV: Packed cells volume, PLT: Platelets, LYM: Lymphocyle, NEUT: Neutrophill

respectively) of PEE, EthE and EAE was observed for the ipsilateral paws. The maximal effect (97.8% at 100 mg kg^{-1}, 106.4% at 30 mg kg^{-1} and 97.4% at 10 mg kg^{-1}, respectively) of PEE, EthE and EAE was observed for the contralateral paws (Fig. 1a-c).

Paws of dexamethasone and methotrexate-treated group showed a significant (ipsilateral paw: $F_{4,10}$ = 59.83 p<0.0001; $F_{4,10}$ = 8069 p<0.0001, respectively; contralateral paw: $F_{4,10}$ = 721.6 p<0.0001; $F_{4,10}$ =1790 p<0.0001, respectively) reduction of MDA levels with maximal effect of 97.6% at 3.0 mg kg^{-1} and 98.9% at 0.1 mg kg^{-1}, respectively of the ipsilateral paws and a maximal effect of 105.3% at 1.0 mg kg^{-1} and 104.0% at 0.1 mg kg^{-1}, respectively of the contralateral paws (Fig. 1d-e).

Tissue myeloperoxidase (MPO): The CFA group showed a significantly (p<0.0001) high level of MPO. However, PEE, EthE and EAE were able to significantly (ipsilateral paw: $F_{4,10}$ = 18.95 p<0.001; $F_{4,10}$ = 20.19 p<0.0001; $F_{4,10}$ = 20.49 p<0.0001, respectively; contralateral paw: $F_{4,10}$ = 13.29 p<0.001; $F_{4,10}$ = 13.36 p<0.001; $F_{4,10}$ = 13.17 p<0.001, respectively) reduce elevated MPO to normal. This significant reduction (ipsilateral paw: $F_{4,10}$ = 14.20 p<0.001; $F_{4,10}$ = 20.72 p<0.0001, respectively; contralateral paw: $F_{4,10}$ = 11.86 p<0.001; $F_{4,10}$ = 13.59 p<0.001, respectively) in MPO levels was also observed for dexamethasone and methotrexate-treated groups (Fig. 2). The maximal effect (99.1% at 100 mg kg^{-1}, 99.6% at 10 mg kg^{-1}, 99.7% at 30 mg kg^{-1}, respectively) of PEE, EthE and EAE was observed for the ipsilateral paws. The maximal effect (103.3% at 30 mg kg^{-1}, 105.6% at 30 mg kg^{-1}, 99.8% at 10 mg kg^{-1}, respectively) of PEE, EthE and EAE was observed for the contralateral paws (Fig. 2a-c). The ipsilateral paws of dexamethasone and methotrexate-treated group showed maximal effect of 99.4% at 0.3 mg kg^{-1} and 99.8% at 0.3 mg kg^{-1}, respectively. The contralateral paws of dexamethasone and methotrexate-treated group also showed a maximal effect of 106.8% at 1.0 mg kg^{-1} and 99.0% at 0.3 mg kg^{-1}, respectively (Fig. 2d-e).

Tissue superoxide dismutase activity: The rate of inhibition of xanthine generated superoxide, expressed as SOD activity was very low in arthritic group. The PEE, EthE and EAE were able to significantly ($F_{4,15}$ = 6.12 p<0.05; $F_{4,15}$ = 16.19 p<0.0001; $F_{4,15}$ =6.30 p<0.001, respectively) increase SOD activity of the ipsilateral paws, respectively, with maximal effects of 96.2% at 30 mg kg^{-1}, 95.3% at 30 mg kg^{-1}, 93.7% at 30 mg kg^{-1}, respectively. At the contralateral paws, PEE, EthE and EAE were also able to significantly ($F_{4,15}$ = 53.18 p<0.0001; $F_{4,15}$ = 143.3 p<0.0001; $F_{4,15}$ = 93.30 p<0.0001, respectively) increase SOD activity, respectively, with maximal effects of 82.1% at 100 mg kg^{-1}, 79.4% at 30 mg kg^{-1}, 85.2% at 100 mg kg^{-1}, respectively (Fig. 3a-c). Dexamethasone and methotrexate

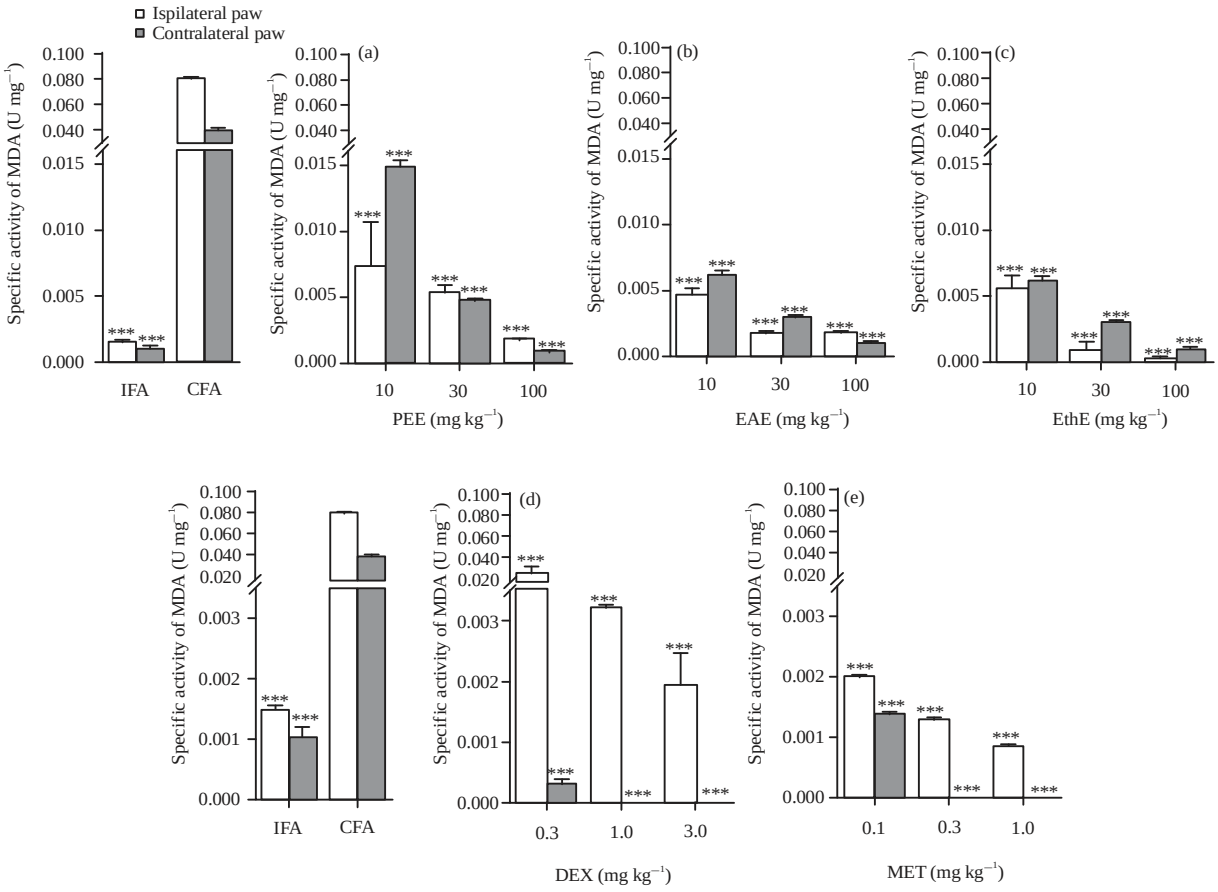

Fig. 1(a-e): Tissue MDA levels in (a) PEE, (b) EAE, (c) EthE-treated groups (d) Dexamethasone and (e) Methotrexate-treated groups for both the ipsilateral and contralateral paws of the arthritic rats. Values are Mean±SEM, (n = 8). ***$p \leq 0.001$ compared to CFA group. All comparison of variances was done using Holm-Sidak's *post hoc* test

likewise ($F_{4,15}$ = 10.00 $p<0.001$; $F_{4,15}$ = 5.04 $p<0.05$, respectively), increased SOD activity of the ipsilateral paws as shown with maximal effects of 96.0% at 3.0 mg kg^{-1} and 95.4% at 1.0 mg kg^{-1}, respectively (Fig. 3d-e). At the contralateral paws, extracts, Dexamethasone and methotrexate were also ($F_{4,15}$ = 56.37 $p<0.0001$; $F_{4,15}$ = 35.52 $p<0.0001$) able to increase SOD activity, with maximal effects of 78.7% at 3.0 mg kg^{-1} and 78.3% at 1.0 mg kg^{-1}, respectively (Fig. 3).

Serum tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6) levels: PEE, EthE and EAE were significant ($F_{3,8}$ = 20.86 $p<0.001$; $F_{3,8}$ = 21.67 $p<0.001$; $F_{3,8}$ = 23.53 $p<0.001$, respectively) in reducing the production of TNF-α with maximal effects of 48.1% at 100 mg kg^{-1}, 47.2% at 30 mg kg^{-1} and 50.3% at 30 mg kg^{-1}, respectively. Dexamethasone and methotrexate dose-dependently ($F_{3,8}$ = 19.15 $p<0.001$; $F_{3,8}$ = 14.50 $p<0.01$, respectively) reduced TNF-α production in arthritic group treated with these drugs, with maximal effect of 50.2% at 3.0 mg kg^{-1} and 51.7% at 1.0 mg kg^{-1}, respectively (Fig. 4).

Evaluating the effects of extracts on IL-6 production, PEE, EthE and EAE were effective ($F_{3,8}$ = 4.39 $p<0.05$; $F_{3,8}$ = 5.61 $p<0.05$; $F_{3,8}$ = 9.56 $p<0.01$, respectively) in reducing the production of IL-6 with maximal effects of 61.0% at 100 mg kg^{-1}, 64.0% at 10 mg kg^{-1} and 71.9% at 100 mg kg^{-1}, respectively. Dexamethasone and methotrexate dose-dependently reduced ($F_{3,8}$ = 6.47 $p<0.05$; $F_{3,8}$ = 7.57 $p<0.05$, respectively) IL-6 production in arthritic group treated with these drugs, with maximal effect of 78.1% at 3.0 mg kg^{-1} and 72.9% at 1.0 mg kg^{-1}, respectively (Fig. 4).

DISCUSSION

Experimental arthritis in animals has some clinical and biochemical features similar to patients with polyarthritic diseases or RA[26]. This model is associated with weight loss due to systemic or local actions of cytokines such as IL-6 and

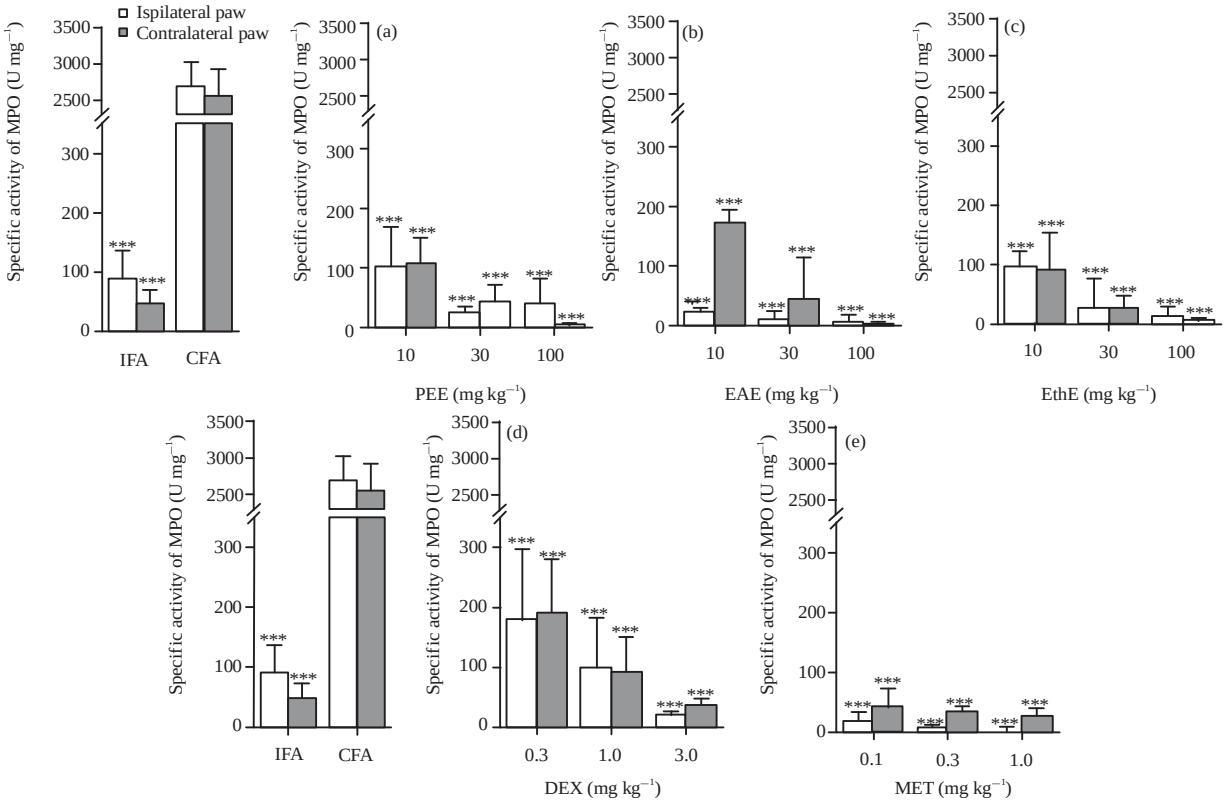

Fig. 2(a-e): Tissue MPO levels in (a) PEE, (b) EAE, (c) EthE-treated groups and (d) Dexamethasone and (e) Methotrexate-treated groups for both the ipsilateral and contralateral paws of the arthritic rats. Values are Mean±SEM, (n = 8). ***$p \leq 0.001$ compared to CFA group. All comparison of variances was done using Holm-Sidak's *post hoc* test

TNF-α[27]. Anaemia (reduction in haemoglobin levels)[28] and oxidative stress[29,30] are also observed. Increased levels of cytokines such as TNF-α, interferon c (INFc), IL-1, IL-6 and IL-17A mRNA have been detected in lymph nodes and/or inflamed joints of rats with adjuvant-induced arthritis, AIA[31,32]. Blockade of these cytokines ameliorates the disease, indicating that these cytokines contribute to the pathology in this model[33-35].

Anaemia is one of the most common systemic manifestation of RA that occurs more during the early stage of the disease[36]. This is mostly related to IL-6 levels which were reported to be significantly higher in patients with anaemia than in persons without anaemia[37]. Also, haemoglobin levels are inversely linked with IL-6 levels[37]. During inflammation, hepcidin, a peptide formed by hepatocytes and a basic iron-regulatory hormone and key mediator of anaemia in patients with chronic disease[38] is induced by IL-6 which rapidly induces hypoferraemia in humans[39]. The hypoferraemia induced results in deformed or inadequate amount of erythrocytes and haemoglobin, resulting in anaemia. Iron release from macrophages in the spleen and iron reuptake in the duodenum is inhibited by plasma hepcidin[38]. Report have shown that serum hepcidin levels are highest in patients with RA and anaemia, with lowest levels reported in healthy adults[40]. Low RBC and Hb concentration of blood of arthritic group confirms with studies that anaemia is associated with arthritis-induced rats and even in patients with RA[41]. Treatment with the extracts reversed the significant decrease in RBC and Hb to normal thus improving the anaemic state of the arthritic rats.

Of the cells involved in inflammation, some (vascular endothelial cells, mast cells and tissue macrophages) are normally present in tissues while others (platelets and leucocytes) gain access from the blood, marked by the movement of phagocytic white blood cells (leucocytes) into the area of injury[42]. The WBC count increases in arthritic rats to destroy invading pathogenic microorganisms and bring about resolution of inflammation[43]. Induration (increased thickness of soft tissue) is due to the accumulation of leucocytes (mostly neutrophils and lymphocytes) and oedema fluid[42]. Reduction of the WBC levels to normal levels, by the extracts could be attributed to the resolution of the inflammatory response.

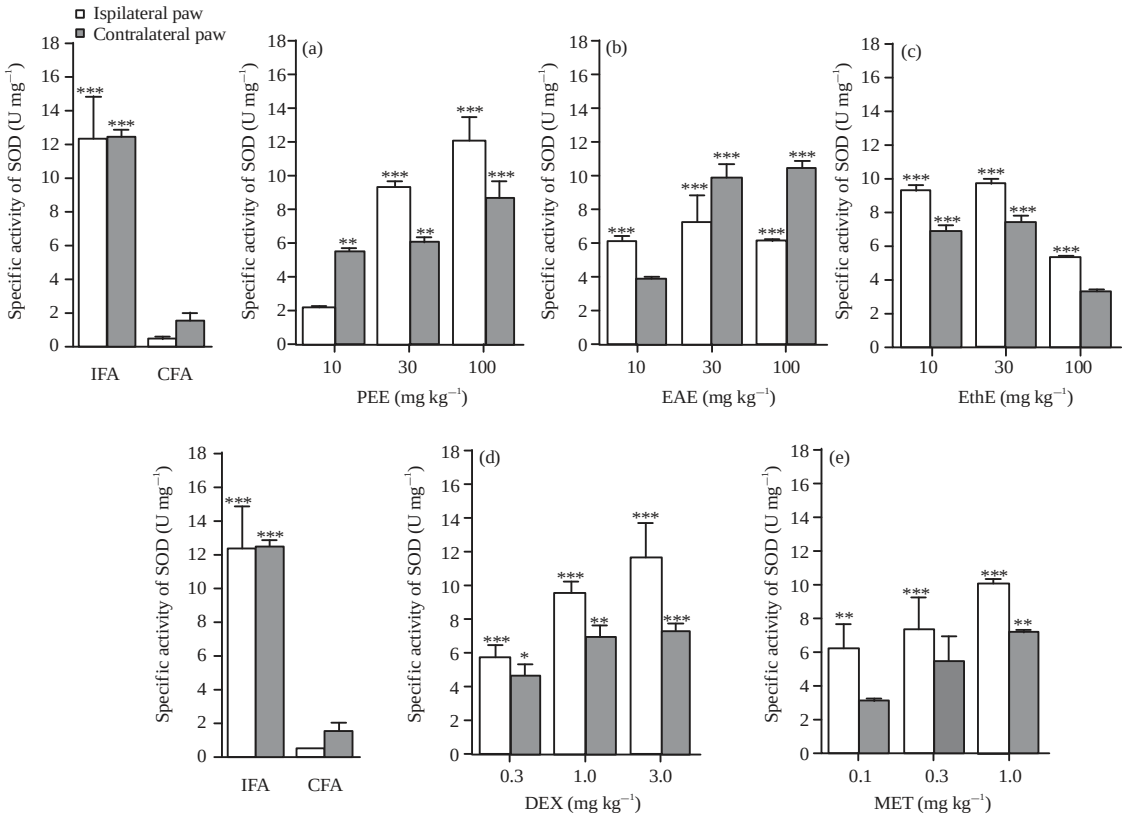

Fig. 3(a-e): Tissue SOD levels in (a) PEE, (b) EAE, (c) EthE-treated groups and (d) Dexamethasone and (e) Methotrexate-treated groups for both the ipsilateral and contralateral paws of the arthritic rats. Values are Mean±SEM, (n = 8). ***$p \leq 0.001$; **$p \leq 0.01$; *$p \leq 0.05$ compared to CFA group. All comparison of variances was done using Holm-Sidak's *post hoc* test

Arthritic rats also had increased platelet count. In the present study migration of leukocytes into the inflamed area was significantly suppressed by the extracts especially PEE at the highest dose. The PEE also significantly decreased WBC count, platelet count and percentage of lymphocytes and neutrophil.

Bone resorption, due to differentiation and activation of osteoclasts, involves a number of cytokines such as TNF-α, IL-1, IL-6. Activated macrophages as well as synoviocytes in the inflamed synovial tissue produces TNF-α which directly and indirectly induces osteoclast formation forming a link between the immune and bone system[44]. From the study the extracts were able to significantly reduce the levels of TNF-α and IL-6 expression. Pro-inflammatory properties of TNF-α include stimulating the production of collagenase and PGE_2 by synovial cells and thus contribute to joint damage. On the other hand, IL-6 acts as a marker for systemic activation of proinflammatory cytokines. It has both proinflammatory and anti-inflammatory properties. Its proinflammatory property is seen as it stimulating the synthesis of acute phase proteins. The anti-inflammatory property of IL-6 is seen as down-regulating the synthesis of IL-1 and TNF-α and inducing the synthesis of glucocorticoids that trigger the synthesis of anti-inflammatory cytokines such as IL-10 and transforming growth factor-β(TGF-β)[27]. The anti-inflammatory property of the extracts contributed so much in reducing the levels of TNF-α and IL-6 expression due to combined activity of phytochemicals present.

Lipid peroxidation is considered a critical mechanism of injury that occurs during arthritis. An indicative method extensively used in evaluating lipid peroxidation is analysis of tissue malondaldehyde (MDA)[45]. The extracts were able to significantly reduce MDA levels in the arthritic tissue comparable to the elevated levels observed in arthritic group. Myeloperoxidase (MPO) a constituent of neutrophil granules reported by Ormrod *et al.*[21] is a marker of infiltration of polymorphonuclear cells which was observed histologically as infiltration of inflammatory cells in the synovial tissue[29] of arthritic group in the study. The study showed that the extracts were able to decrease this infiltration by reducing levels of MPO. The superoxide radicals are the first product of molecular oxygen reduction. The enzyme, superoxide dismutase[23] acts as a catalyst for dismutation of superoxide

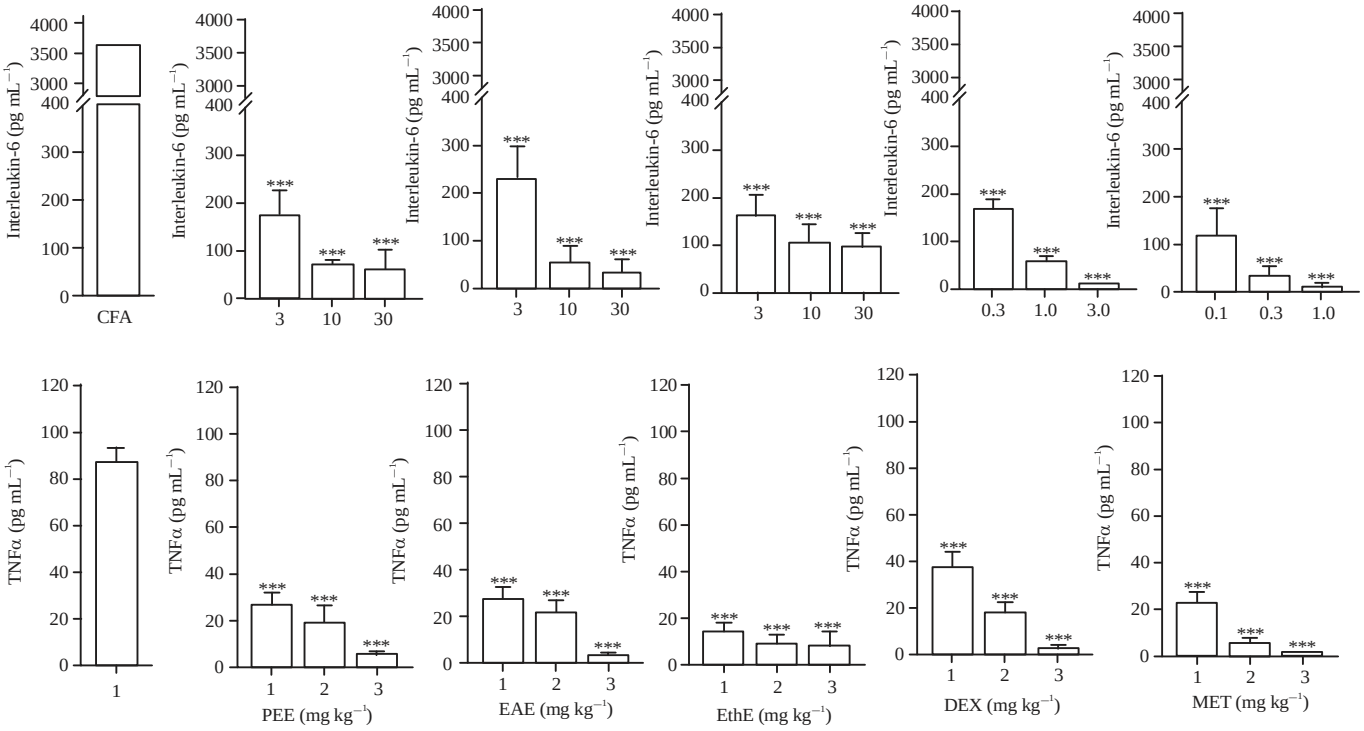

Fig. 4(a-b): Serum (a) TNF-α and (b) IL-6 production in PEE, EthE and EAE, dexamethasone and methotrexate-treated groups of the arthritic rats. Values are Mean±SEM, (n = 8). ***p≤0.001 compared to CFA group. All comparison of variances was done using Holm-Sidak's *post hoc* test

radicals into H_2O_2 and into molecular oxygen to protect cells and tissues from superoxide radicals and other peroxides such as lipid peroxides *in vivo*[30]. In the arthritic group, SOD activity was markedly reduced. This decrease can be as a result of inhibition of the enzyme by hydrogen peroxide, indicating a high degree of superoxide anion production. High levels of superoxide anion in the tissue suggest increased hydrogen peroxide liberation through dismutation reaction. Increased in enzyme activity observed in the extracts-treated groups suggest that there was an adaptive response of the animal against possible damage caused by oxygen free radicals. The extracts can thus be said to have antioxidant effect, hence its ability to inhibit lipid peroxidation through the decrease in neutrophil accumulation and decrease in the chemotactic reduction of peroxide.

The anti-inflammatory effects of the extracts of this plant could be attributed to some phytochemicals present. Previous study indicated the presence of alkaloids, terpenoids, phytosterols and reducing sugars in all the extracts studied. EAE and EthE also showed the presence of tannins, cardiac glycosides, anthraquinones and saponins[46]. Inflammation induced by several models such as have been used to demonstrate the anti-inflammatory effects of many alkaloids have been demonstrated using several models such as; carrageenan-induced pedal oedema, collagen II -induced arthritis, 5-HT-induced pedal oedema, xylene-induced ear oedema, TPA-induced inflammation, acute inflammation induced by *E. coli* LPS and TNB-induced colitis among others[47]. Terpenoids and cardiac glycosides can modulate critical cell signaling pathways involved in inflammatory response such as nuclear transcription factor-kappaB (NF-kappaB) activation[48,49], significant inhibition cytokine release and inhibition of T-cell immune responses[50]. Tannins (hydrolysable and non-hydrolysable) are reported to have anti-inflammatory activity in carrageenan and dextran-induced rat paw oedema, cotton pellet granuloma test and adjuvant-induced polyarthritis in rats, possibly by antagonism of the permeability-increasing effects of some inflammatory mediators, therefore inhibiting the migration of leucocytes to an inflammatory site and further spread of mediators[51]. Anthraquinones also possess anti-iflammatory activity by inhibiting NO production, cyclooxygenase-2 (COX-2) and prostaglandin E_2 (PGE_2) in a LPS-induced inflammation model and in carrageenan-induced paw oedema[52]. Alkaloids are reported as reducing elevated levels of proinflammatory cytokines[53].

CONCLUSION

The petroleum ether, ethyl acetate and ethanol extracts of *Trichilia monadelpha* stem bark improved haematological features, decreased MPO, MDA, TNF-α and IL-6 levels and increased SOD activity. This indicates its potent anti-inflammatory and antioxidant property making it very useful in the traditional management of arthritis.

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