INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease of the joints that leads to changes in bone metabolism [1]. The primary target of this inflammatory disease is the synovial membrane of the joints. RA leads to cartilage and bone erosion and joint deformity; manifesting signs and symptoms such as pain, swelling and redness, and fatigue [2]. It can affect other organs of the body resulting in disability and mortality [3]. Globally, RA affects about 1% of adult population [4] and poses a heavy economic burden with disease progression [5].

Non-steroidal anti-inflammatory drugs (NSAIDs), disease-modifying antirheumatic drugs (DMARDs), corticosteroids, and cytokine-targeted drugs have been used to manage RA [6,7]. These drugs target specifically at reducing inflammation and relieving pain by blocking cyclooxygenase-mediated prostaglandins release (i.e., the NSAIDs) and control of joint inflammation by suppressing inflammatory-induced bone erosion (glucocorticoids and DMARDs) [8,9]. However, these drugs are associated with undesirable side effects such as gastric ulceration and precipitation of asthma and renal disease and are unaffordable to a lot of individuals [10], hence this search for natural products that are efficacious, but with less

Effect of Trichilia monadelpha (Meliaceae) extracts on bone histomorphology in complete Freund’s adjuvant-induced arthritis

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ABSTRACT

Aim: This study aimed to assess the effect of petroleum ether extract (PEE), ethyl acetate extract (EthE), and ethanol extract (EAE) of Trichilia monadelpha stem bark on bone histomorphology in arthritis.

Methods: Percentage inhibition of edema and arthritic scores in complete Freund’s adjuvant-induced (0.1 ml of 5 mg/ml of heat-killed Mycobacterium tuberculosis in paraffin oil-injected subplantar into the right hind paw) arthritic Sprague-Dawley rats treated with PEE, EthE, or EAE (10,30, and 100 mg/kg, respectively), dexamethasone (0.3-3.0 mg/kg), or methotrexate (0.1-1.0 mg/kg) over a 28-day period were estimated. Rat paws were radiographed and scored. Body weights were taken and paw tissues were harvested for histopathological studies.

Results: The extracts significantly (P ≤ 0.01-0.0001) and dose dependently reduced the polyarthritic phase of arthritis. EAE and PEE significantly (P ≤ 0.01-0.0001) minimized edema spread from acute arthritic phase (days 0-10) to polyarthritic phase (days 10-28). EthE improved which deteriorated body weight in arthritis. All extracts significantly (P ≤ 0.05-0.01) improved arthritic score; reducing erythema, swelling and joint rigidity, and also significantly (P ≤ 0.05-0.01) reduced hyperplasia, pannus formation, and exudation of inflammatory cells into synovial spaces. Conclusion: The stem bark extracts of T. monadelpha reduce bone tissue damage and resorption associated with adjuvant-induced arthritis, hence could be useful in managing arthritis in humans.

KEY WORDS: Arthritic paw radiography, erythema, subchondral erosion, periostitis, osteolysis, Trichilia monadelpha
side effects, and affordable to the majority of the populace. *Trichilia monadelpha* (Meliaceae), one such plant, has been used trado-medically to manage chronic inflammatory diseases for decades [11,12]. The stem bark has anti-inflammatory [13] and analgesic effects [13,14]. It improves sperm viability [15] and is relatively non-toxic [13]. Phytochemical analysis conducted revealed the presence of alkaloids, terpenoids, phytosterols, coumarins, tannins, cardiac glycosides, anthraquinones, saponins, flavonoids, and reducing sugars in the petroleum ether, ethyl acetate, and hydroethanolic extracts [13,16].

This study, therefore, assesses the effects of petroleum ether extract (PEE), ethyl acetate extract (EthE), and ethanol extract (EAE) of *T. monadelpha* stem bark in complete Freund's adjuvant (CFA)-induced 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 aluminum cages and given normal rat diet (Agricare Ltd., Tanoso, Kumasi) and water to consume *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). The study was approved by the Institutional Review Board on animal experimentation.

**Extract Preparation and Dosing**

A voucher specimen of extraction of the stem bark of *T. monadelpha* was kept in the herbarium, Faculty of Pharmacy (No. FP/079/10) as described by Ben et al., 2013 [16]. The PEE, EthE, and EAEs were each triturated with Tween-80 (5 drops) in normal saline and administered orally to rats at doses ranging from 10 to 100 mg/kg.

**Drugs and Chemicals used**

Heat-killed *Mycobacterium tuberculosis*, mixed strains C, DT, and PN (Ministry of Agriculture, Fisheries and Food, U.K) were used to induce arthritis. Dexamethasone (DEX) (Wuhan Grand, China) and methotrexate (MET) (Dabur Pharma, India) were the reference drugs used for the treatment of arthritis.

**CFA-induced Arthritis**

Adjuvant arthritis was induced as previously described by Pearson, 1956 [17], with modification according to Woode et al., 2008 [18]. In brief, rats were assigned to 17 groups (n=8) and injected with a 0.1 ml suspension of CFA (5 mg/ml of heat-killed *M. tuberculosis* in paraffin oil) into the right hind paw. Arthritic control group received only subplantar injection of CFA, while non-arthritic control/incomplete Freund’s Adjuvant (IFA) group received only subplantar injection of 0.1 ml IFA (sterile paraffin oil). PEE, EthE, or EAE (10, 30, and 100 mg/kg p.o.), DEX (0.3-3.0 mg/kg i.p.) or MET (0.1-1.0 mg/kg i.p.) were administered to rats in the various groups, respectively, after establishment of arthritis, i.e., on day 10 [Table 1].

Ipsilateral (injected) and contralateral (non-injected) paw volumes were measured using a water displacement plethysmometer (IITC Life Science Equipment, Woodland Hills, USA). This was before subplantar injection of CFA (day 0) and every other day for 28 days after subplantar injection of CFA and IFA. Data obtained for ipsilateral and contralateral paw volumes were individually recorded as percentage of change from their values at day 0 and then averaged for each treatment group. These were presented as the effect of drugs on the time course and the total edema response of adjuvant-induced arthritis for the 28-day period. Total paw volume for each treatment was calculated in arbitrary unit as area under the curve (AUC) to determine the percentage inhibition as per the formula below:

\[
\%
\text{Inhibition of edema} = \left( \frac{AUC_{control} - AUC_{treatment}}{AUC_{control}} \right) \times 100
\]

The initial body weight and arthritic score of rats were taken on day 0 after grouping and every other day for 28 days of the experiment, followed by subplantar injection of 0.1 ml CFA. Radiographic images of the paws were taken. Paw tissues were harvested after the 28th day for histopathological assessment.

**Body Weight and Arthritic Score**

Body weight and arthritic scores were recorded for each hind joint and the tail by a consistent observer blinded to the treatment received by the animals. Scoring was performed on a 0-5 scale [Table 2].

**X-ray Radiography**

On day 28, the animals were anesthetized by intraperitoneal injection of 20 mg/kg pentobarbitone. Radiographic images of the hind limbs were taken using a Faxitron X-ray machine (Hewlett-Packard, Buffalo Grove, IL) with a 0.5 mm focal spot.

| Group 1 | Non-arthritic control/IFA (subplantar injection of 0.1 ml IFA) |
|---------|---------------------------------------------------------------|
| Group 2 | Arthritic control/CFA (subplantar injection of 0.1 ml of CFA) |
| Groups 3-5 | Treated with DEX (0.3, 1.0, and 3.0 mg/kg i.p.) from day 9 and administered every other days |
| Groups 6-8 | Treated with MET (0.1, 0.3, 1.0 mg/kg i.p.) from day 9 and administered every 4 days |
| Groups 9-17 | Treated with extracts (10, 30, and 100 mg/kg p.o.) from day 9 and administered every day |

**Table 1: Experimental grouping and treatment in adjuvant-induced arthritis**

CFA: Complete Freund’s adjuvant.
beryllium window, and X OMAT TL (onscreen) film. The focal film distance was 61 cm, and exposures were made over 30 s at 45 kVp and 3 mA. Radiographs were analyzed by a board-certified radiologist who was blinded to the treatment groups. Quantitative scores were generated for radiographic changes in the joints in the following areas: Soft tissue volume, joint space, subchondral erosion, periostitis, osteolysis, subluxation, and degenerative joint changes. The values were based on increasing severity of demineralization [Table 3].

**Histopathology**

Hind limbs of arthritic rats were removed and fixed in 10% buffered formalin. The limbs were decalcified in 5% formic acid, processed for paraffin embedding, sectioned at 5 µm thickness, and later stained with hematoxylin and eosin for examination under a light microscope. The histopathological change of joints was blindly graded by a pathologist and assigned a score of 0-3 [Table 4].

**Statistical Analysis**

Statistical analysis of data was done using Sigma Plot version 12.3 (Systat Software Inc. Chicago USA). Significant differences in paw volumes, body weight, arthritic scores, and AUCs for parameters measured were ascertained using 1-way and 2-way analysis of variance and Holm-Sidak’s post hoc test. Values plotted were mean ± SEM. P ≤ 0.05 and higher F values (P ≥ 4.0) were considered statistically significant.

**RESULTS**

**CFA-induced Arthritis**

CFA injection into the paws of rats produced a biphasic response observed as paw swelling or edema of the ipsilateral and contralateral paws. The first phase is the acute phase, characterized by unilateral edema of the ipsilateral paws. The subsequent phase, polyarthritic/chronic phase, is characterized by edema of the contralateral paws. All arthritic control animals showed acute inflammatory edema at the ipsilateral (injected paws) paws around days 9-10 followed by subsequent chronic polyarthritic phase which begins around days 10-12. Throughout the 28-day experiment, there was no significant change in the paw volume of the non-inflamed control groups injected with IFA.

**Acute-phase Inflammation**

PEE, EthE, EAE, DEX, and MET significantly (P ≤ 0.01-0.0001; F < 3.28 = 5.57-57.76) reduced acute-phase inflammation [Figure 1a, c and e; Figure 2a and c]. However, while PEE and EAE ameliorated the edema in the ipsilateral paws by 92.7% and 76.2%, respectively, at 100 mg/kg, EthE effect was not significant (38.8% at 100 mg/kg) [Figure 1b, d and f]. DEX and MET significantly ameliorated edema in the ipsilateral paws, i.e., 100% inhibition at 3 mg/kg and 80.0% inhibition at 1 mg/kg, respectively [Figure 2b and d].

**Polyarthritic/Chronic-phase Inflammation**

PEE, EthE, EAE, DEX, and MET significantly (P ≤ 0.01-0.0001; F < 3.28 = 5.57-57.76) reduced polyarthritic/chronic-phase inflammation [Figure 1a, c and e; Figure 2a and c]. PEE, EAE, DEX, and MET also significantly (P ≤ 0.01-0.0001; F < 3.28 = 4.69-10.43) minimized the progression of the inflammation from the acute to the polyarthritic phases, [Figure 1b and f; Figure 2b and d]. PEE, EthE, and EAE ameliorated the edema in the contralateral paws, with inhibitory effects of 98.0, 69.1, and 70.8%, respectively, at 100 mg/kg [Figure 1b, d and f]. DEX and MET also caused 125.3% inhibition at 3 mg/kg and 94.7% inhibition at 1 mg/kg, respectively [Figure 2b and d]. PEE and EAE showed greater potency and efficacy comparable to DEX and MET [Table 5].

**Body Weight**

The CFA group experienced weight loss with excess swelling, erythema, and joint rigidity in both ipsilateral and contralateral
Arthritic Score

PEE, EthE, and EAE significantly reduced ($P < 0.05-0.01$; $F = 3.77-5.77$) arthritic score. The effect was, however, not comparable to DEX and MET ($P < 0.0001$; $F_{3,28} = 13.34-32.16$), which were more potent [Figure 4a-c].

X-ray Radiography

Radiographs of rats from CFA group displayed arthritic changes characterized by soft-tissue swelling with bone demineralization occurring mostly at the tibiotarsal joint which are indications...
of bone damage, in both ipsilateral and contralateral paws, compared with IFA group, which had intact bone structure. Radiographs of rats from PEE, EthE, and EAE treatment resulted in a dose-dependent reduction ($P \leq 0.01-0.001; F_{4,9} = 6.99-65.14$) in inflammation with the morphology of the synovium looking normal [Figure 5a, b and c]. DEX and MET improved the morphology of the tissue significantly ($P \leq 0.01; F_{4,9} = 17.75-18.75$) [Figure 5d and e].

**DISCUSSION**

Adjuvant-induced arthritis in rats is an experimental model for therapeutic and pathogenetic studies of chronic forms of arthritis [19-22]. Chronic arthritis is usually associated with bone loss, due in part to systemic or local actions of interleukin (IL)-6 and tumor necrosis factor-α (TNF-α) [20]. These
cytokines stimulate the release of tissue-destroying matrix metalloproteinases as well as by inhibiting the production of endogenous inhibitors of these metalloproteinases, the net result being joint damage. Pathological features of this disorder include edema, infiltration of mononuclear and polymorphonuclear cells into the joint (synovial spaces), pannus formation, periostitis, and erosion of cartilage and bone [21].

In this study, adjuvant-injected paw was typified by a rapid onset of inflammation evident within 24 h of adjuvant injection, which continued to increase up to day 21 post-induction. This allowed for the study of acute inflammatory reactions locally (at the site of injection), i.e., in the ipsilateral paw, as well as the immunological reaction that develops later in the contralateral paw and various organs [22]. The arthritic rats showed soft-tissue swelling around the ankle joints during arthritis, and it was considered edema of those particular tissues. As the disease progressed, a more diffused demineralization developed in the extremities [23]. This was observed from the X-ray of the control group. Secondary lesions of adjuvant arthritis occurred after a delay of approximately 10 days and were characterized by inflammation of non-injected sites (right hind legs, ears, and tail) and further increases in the volume of the injected hind leg.

A therapeutic treatment regimen was followed in this research by initiating treatment from day 10 to day 28. All treatments with extracts, especially PEE and EAE, were effective in reducing the primary edema by day 18. The non-injected paw developed secondary lesion by day 14 post-adjuvant injection as a result of immune response to the bacterial adjuvant [17]. Treatment of adjuvant-injected rats with the extracts showed a significant reduction of secondary paw inflammation (compared with arthritic controls). These observations suggest that the extracts have very significant anti-inflammatory activity, comparable to DEX and/or MET. These drugs (DEX and MET) target specifically two major aspects, namely reducing inflammation and relieving pain by blocking cyclooxygenase (COX)-mediated prostaglandins release and control of joint inflammation by suppressing inflammatory-induced bone erosion. This finding is in line with earlier publications which indicate that preparations of the stem bark of *T. monadelpha* have been used in Ghanaian traditional medicine to treat pain and inflammation for many years and their efficacies are widely acclaimed in different communities in Ghana [13,14,24-26].

Earlier phytochemical screening conducted revealed the presence of alkaloids, terpenoids, phytosterols, and reducing sugars in all the extracts studied. EAE and EthE also contained...
tannins, cardiac glycosides, anthraquinones, and saponins [16]. The anti-inflammatory activity exhibited by the extracts could be attributed to the combined effects of some of these phytochemicals. Many alkaloids have been ascertained to have anti-inflammatory activity using in vivo models such as carrageenan-induced pedal edema, 5-HT-induced pedal edema, xylene-induced ear edema, among others, and in vitro model including inhibitory activity on COX-1 and COX-2 and inhibitory activity on prostaglandin E₂ (PGE₂) and NO production [27]. This indicates diverse mechanisms by which alkaloids exert anti-inflammatory effect. Terpenoids and cardiac glycosides have the ability to modulate critical cell signaling pathways involved in the inflammatory response of the body such as nuclear transcription factor-kappa B activation [28,29], significant inhibition of cytokine production, and inhibition of T-cell immune responses among others [30]. A mixture of tannins (hydrolysable and non-hydrolysable) have been demonstrated to have apparent anti-inflammatory activity in carrageenan- and dextran-induced rat paw edema, cotton pellet granuloma test, and adjuvant-induced polyarthritis in rats. It is thought to be due to antagonism of the permeability-increasing effects of some inflammatory mediators, thus inhibiting the migration of leukocytes to an inflammatory site [31]. Anthraquinones were also found to possess anti-inflammatory activity after their inhibitory activities on NO production, COX-2, and PGE₂ which was determined in a lipopolysaccharide-induced inflammation model and in carrageenan-induced paw edema [32]. Reduction of paw swelling from the 3rd week onward may have been due to immunological protection rendered by the plant extracts, preventing systemic spread and ultimately reducing the destruction of joints as seen in the arthritic scores for the photographs and the radiographs. The phytochemicals in the extracts could have contributed to the immunological protection due to their significant anti-inflammatory properties which suppresses the generation and spread of pro-inflammatory agents [27]. Reduced bone structure and increased re-absorption cause bone loss in adjuvant-induced arthritis in rats [33,34]. Results of radiographic scores clearly showed increased bone loss in arthritic groups. The extracts, especially PEE, and reference drug treatment decreased bone loss due to arthritis. This suggests a suppression of synovitis and protection of bone structure resulting in joint protection [9,35,36]. This effect conforms to one of the therapeutic strategies of managing arthritis. The major target for inflammatory process in adjuvant-induced arthritis is the synovium which results in tissue inflammation as a result of infiltration of the tissue with multiple immune cells and cytokines [37]. The tissue inflammation is observed.
as expansion of the synovial tissue and pannus formation that invades the bone and cartilage, destroying the tissue as it proceeds [38]. This process promotes osteoclastogenesis that leads to focal articular bone erosion at the site of pannus formation, as well as systemic bone loss similar to osteoporosis [38,39]. Inflammatory tissue invasion, into the subchondral bone, results in involvement of many cell types such as fibroblasts, lymphocytes, and monocytes [37]. Monocytes are the precursors of osteoclasts which bring about reabsorption of bone through the acidic dissolution of bone mineral and enzymatic destruction of bone matrix. This reabsorption of the bone by osteoclasts is due to the synthesis of proteases by the synovial fibroblasts, neutrophils, and the chondrocytes.

Chronic arthritis is usually associated with weight loss. This may be due to the systemic or local action of inflammatory cytokines such as TNF-α and IL-1β [20,40] produced primarily by monocytes and macrophages [41]. The high concentrations of TNF-α and IL-1β exert a powerful influence on whole-body protein and energy metabolism. Although the specific mechanism(s) is not known, TNF-α is thought to stimulate muscle catabolism [42]. The increased catabolism raises energy expenditure, which leads to weight loss and reduced lean body mass, especially if energy and protein requirements are not met; a phenomenon recognized as “rheumatoid cachexia.” Changes in body weight, therefore, have also been used to assess the course of disease and response to therapy of anti-inflammatory drugs [43]. The extracts significantly improved body weight of arthritic rats, indicating a reduction of cachexia caused by the inflammatory cytokines and hence their therapeutic potential in the management of RA.

CONCLUSION

This study has demonstrated that PEE, EthE, and EAEs of the stem bark of *T. monadelpha* have interesting antiarthritic property by reducing bone tissue damage and resorption in Freund’s complete adjuvant-induced arthritis and hence the extracts are worth further investigating as they could be very useful to humans.

ACKNOWLEDGMENTS

We are grateful for the technical support of the Department of Pharmacology, CHS, KNUST, and the X-ray and Ultrasound Department of the KNUST Hospital, Kumasi, Ghana.

Table 6: Arthritic scores of arthritic paws (ipsilateral and contralateral) obtained by X-ray radiography after CFA-induced arthritis and treatment with *T. monadelpha* stem bark extracts and reference drugs

| Groups | Doses | Arthritic score |
|--------|-------|-----------------|
|        |       | Ipsilateral paws | Contralateral paws |
| IFA    | 0±0.0** | 0±0.0** |
| CFA    | 4±0.3  | 4±0.3 |
| PEE    | 10 mg/kg₁ | 2±0.9 | 2±0.3 |
|        | 30 mg/kg₁ | 2±0.7 | 0.7±0.7 |
|        | 100 mg/kg₁ | 1±0.6 | 1±0.3 |
| EthE   | 10 mg/kg₁ | 3±0.6 | 2±0.3 |
|        | 30 mg/kg₁ | 2±0.9 | 2±0.9 |
|        | 100 mg/kg₁ | 4±0.3 | 2±0.9 |
| EAE    | 10 mg/kg₁ | 3±0.3 | 2±1.0 |
|        | 30 mg/kg₁ | 3±0.6 | 1±0.7 |
|        | 100 mg/kg₁ | 2±0.9 | 2±0.9 |
| DEX    | 0.3 mg/kg₁ | 1±0.9 | 0.7±0.7 |
|        | 1.0 mg/kg₁ | 0.0±0.0** | 0.3±0.3* |
|        | 3.0 mg/kg₁ | 0.0±0.0** | 0.0±0.0** |
| MET    | 0.1 mg/kg₁ | 0.7±0.3 | 0.3±0.3* |
|        | 0.3 mg/kg₁ | 1±0.6 | 0.7±0.7 |
|        | 1.0 mg/kg₁ | 0.3±0.3* | 0±0.0** |

Values are mean±SEM. ***P≤0.001, **P=0.01, *P≤0.05 ANOVA followed by Holm-Sidak’s post hoc test. PEE: Petroleum ether extract, EthE: Ethyl acetate extract, EAE: Ethanol extract, DEX: Dexamethasone, MET: Methotrexate, IFA: Incomplete Freund’s adjuvant, CFA: Complete Freund’s adjuvant, T. monadelpha: Trichilia monadelpha, ANOVA: Analysis of variance

Figure 5: Histopathological score for bone erosion, inflammatory cell infiltration, pannus formation, synovial hyperplasia, and fibrosis of arthritic rats treated with (a) the petroleum ether extract, (b) the ethyl acetate extract, (c) the ethanol extract of *T. monadelpha* stem bark, (d) dexamethasone and (e) methotrexate incomplete Freund’s adjuvant, complete Freund’s adjuvant. Bars plotted represent mean scores±SEM (n=8). ***P≤0.001; **P≤0.01; *P≤0.05 compared to complete Freund’s adjuvant group. (one-way analysis of variance followed by Holm-Sidak’s post hoc test)
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