Original Research Article

EVALUATION OF ANTIARTHRITIC PROTECTIVE EFFECT OF *ERLANGEA TOMENTOSA* (OLIV. &HIERN) S. MOORE (ASTERACEAE) METHANOL LEAF EXTRACT IN COMPLETE FREUND'S ADJUVANT-INDUCED ARTHRITIS IN RATS

ABSTRACT

Objective: The goal of this study was to investigate the antiarthritic effects of *Erlangea tomentosa* methanol leaf extract in CFA-induced arthritis in rats.

Methodology: The plant extract was administered to arthritic rats that had been injected with the Complete Freund's adjuvant (CFA). The physical anti-arthritis effects of the plant were evaluated using body weight, paw diameter, and a clinical scoring approach. The systemic anti-arthritis properties were evaluated using hematological and biochemical markers of arthritis at the conclusion of the study. Paw joints were taken out for histopathological investigation, and the body weight organ indices for gross abnormalities.

Results: It was found that the methanol leaf extract of the plant significantly reduced the arthritis state, which was obvious in clinical arthritis scoring, CFA-injected paw size, body weight, and the preservation of hematological and biochemical indicators. Histopathology and organ indices also confirmed that the plant extract could reduce the pain and inflammation of animals with CFA-induced arthritis.

Conclusion: The authors have concluded that the plant might help with arthritis. Research into a specific proinflammatory mediator, such as interleukins and tumor necrosis factor, would confirm this activity.

Keywords:Complete Freund's adjuvant (CFA), Erlangea tomentosa, Antiarthritis activity, Methanol leaf extract

1. INTRODUCTION

The term arthritis refers to joint inflammation (originating from the Greek words "artho-," which means joint, and "-itis," which means inflammation). Arthritis is a series of medical conditions collectively referred to as "arthritis"[1–2]. As a result, arthritis is a disease wherein one or more of the body's joints become inflamed. Trauma, infections, autoimmune diseases, undiagnosed reasons, and aging are only a few of the causes [2–3]. Arthritis is often classified into more than a hundred types. The more popular forms of arthritis are ankylosing-spondylitis (AS), rheumatoid arthritis (RA), osteoarthritis (OA), adolescent-arthritis, psoriatic arthritis (PsA), as well as gouty-arthritis (GA). However, RA is the most frequent of these various types of arthritis [4].

RA is a progressive auto-immune illness that causes symmetrical inflammatory poly-arthritis in the feet and hands, progressing to the wider joint surfaces [5]. Pressure, stiffness, and swelling are common articular symptoms. Deformation and loss of function of affected joints result from the gradual deterioration of articular cartilage. The synovium is the

primarily affected region, particularly at the site where the "pannus," a mass of abnormal tissue, develops [6] [7]. RA may cause lacerations in organs and tissues exterior to the articulations, like the heart and lungs, as well as blood vessels, making it a highly debilitating disease. In RA, the risk of developing atherosclerosis and lymphoma is increased [8]. Cardiovascular illness (31%), pulmonary fibrosis (4%), as well as lymphoma (2.3%), according to a 2007 report, all have higher mortality rates among people with RA [9].

Inflammatory processes in different joints change significantly in the general pathophysiology of RA. Inflammatory mediators, autoantibodies, cytokines, growth factors, chemokines, adhesion molecules, as well as matrix-metalloproteinases (MMPs), among others, all promote the development of this systemic and chronic disease. The propagation of the disease is aided by these mediators and immune cells [10]. As a result, inhibiting pathological hyperplasia, inflammatory cytokines, as well as matrix metalloproteinases in synovial cells is critical for treating RA synovitis [5]. There is no single laboratory test that can validate whether or not an individual has RA [8]. The treatment is then directed using a mixture of clinical, laboratory, as well as imaging tools. Inflammatory markers, hematological, biochemical, and autoimmune variables are examples of laboratory tests that might be useful for people who have a strong suspicion that they have RA [11].

Current treatments are costly and less successful because of side effects, and the need for new medicines, especially from natural sources, is required [7–12].*E. tomentosa*(Oliv. & Hiern) (Asteraceae) is an herb plant commonly found in Uganda known as Ekyoganyanja in Runyankore. It is traditionally used to manage various illnesses by the locals, including colic pains, stomachaches, syphilis, fever, cough, anemia, conjunctivitis, among others [13–14]. A recent study reported *E. tomentosa* to have potent analgesic and anti-inflammatory effects in mice and rats, respectively [15].

Based on this scientific evidence, this project was planned to look into the antiarthritic effects of *E. tomentosa* methanol leaf extract in chronic conditions like RA in rats with CFA-induced arthritis.

2. MATERIAL AND METHODS

2.1 Study design

This was an in vivo experimental study. The plant methanol leaf extract was tested for its antiarthritic effects using Complete Freund's Adjuvant (CFA)-induced arthritis in rats.

2.2 Collection, identification, and extraction of plant material

The leaves were collected from Bwegiragye village, in Bushenyi District, Western Uganda. The plant was identified by a plant taxonomist from Mbarara University of Science and Technology (MUST). A voucher specimen with the number B50891 was deposited in the herbarium of the Department of Botany for future reference. The plant extracts were prepared according to Begashaw and Porwal's approaches [16–17]. To summarize, newly collected plant leaves were washed with tap water to eliminate adherent particles before being dried in the shade until a consistent mass was achieved. With the aid of a mortar and pestle, the dried leaves were ground into a fine powder. To obtain the hydroalcoholic crude extract, we macerated this coarsely powdered plant (using a 1g/10ml ratio) in 70% methanol for 3 days at room temperature in an Erlenmeyer flask. Filter paper was used to remove the filtrate from the marc after 72 hours (Whatman No.1). The solvent was then enabled to vaporize from the filtrate using a rotary evaporator under reduced pressure and more concentrated in a water bath at 55 ◦C. The condensed extract was maintained at 4 °C till the experiment was over [17].

2.3 Experimental animals

The animals of 8-10weeks used for the experiment were obtained from the animal house facility of the Department of Pharmacology and toxicology, Kampala International University Western Campus (KIU-WC). Animals of the same gender were randomly allocated into the control and treatment groups. They could be housed separately if it was technically necessary. The rats were kept in their havens for five days prior to the start of the treatment study to permit

acclimatization to the laboratory environments. The rats were fed a normal rodent pellet diet and had access to unlimited water [18].

2.4 Test chemicals and solvents

The diclofenac sodium tablets (BP 50 mg), halothane (4%), filter paper, and methanol of analytical grade (99.5%) used in this study were purchased at a local chemical shop supplier, Ltd. (Bushenyi District). The CFA used was obtained from Sigma-Aldrich.com [1], batch no. #SLBP043IV.

2.5 Arthritis induction

To induce arthritis in rats, 0.1ml of CFA was injected subcutaneously into the right hind-paw as recommended by the AIA protocol [21]. The main visible signs of arthritis (redness, swelling, edema, or erythema) were seen to prove that the arthritis induction process was working.The adjuvant is safe paraffin oil (5mg/ml) comprising *Mycobacterium tuberculosis* that has been heat-killed [22].

2.6 Experimental setup

Thirty male Wistar albino rats were arbitrarily allocated into six groups of five rats each. Arthritis was triggered thirty minutes (30) after the vehicle or drug has been administered orally. For extract, standard drugs, and vehicle administrations, the oral route was used. The animals were treated for twenty-one days as follows:

Group 1: Animals served as normal control (without CFA-injection and no treatment).
Group 2: Animals received a single-injection of 0.1 mL of 0.5 % CFA with no treatment (Arthritic control).
Group 3 animals received a single-injection of 0.1 ml of 0.5 % CFA and treated with 1.5 mg/kg of diclofenac (Positive control).
Group 4: Animals received a single-injection of 0.1 ml of 0.5 % CFA and treated with 75mg/kg plant extract.
Group 5: Animals received a single-injection of 0.1 ml of 0.5 % CFA and treated with 150mg/kg of the extract.
Group 6: Animals received a single-injection of 0.1 ml of 0.5 % CFA and treated with 300mg/kg of the extract.

On days 0, 7, 14, and 21, the body weight of the rats was weighed and recorded using the analytical weighing balance, and the right paw sizes were also measured using Vernier caliper. On day 22, the arthritic scores were determined basing on paw size clinical appearance. Finally, the animals were euthanized while inhaling 4% halothane gas to look at joint histopathology, as well as specific hematological, and biochemical parameters [23].

2.6.1 Arthritic clinical scoring approach

The following visual criteria were used to monitor the arthritis morphological characteristics of redness, swelling, and erythema: normal-paw = 0, mild-swelling and erythema of the digits = 1, swelling and erythema = 2, severe-swelling and erythema = 3, gross deformity, as well as incapability to utilize the limb = 4 [24].

2.6.2 Bodyweight and hind paw size method

The diameter of all rats' hind paws was measured using a Vernier caliper on the first day (0^{th} day) before CFA administration and then weekly until the 21^{st} day (i.e., 0, 1, 4, 7, 14, and the 21^{st} day). By deducting the terminal paw-size from the starting diameter, the hind paw size could be calculated. Animals' body mass was documented weekly from the 0^{th} day until the completion of the experiment. The discrepancy between the initial and final weight of animals could be used to measure body weight changes [12]. For different weeks, the mean increases in inoculated paw edema for initial paw size (PS) were determined, and the percent inhibition of paw edema for the untreated category was computed using the method below [25].

%inhibition = [(PS of arthritic control-PS of treatment control)/ PS of arthritic control] X 100

2.6.3 Hematological, and biochemical parameters

Using common laboratory procedures, specific hematological markers, such as red blood cell (RBC) and white blood cell (WBC) counts, hemoglobin (Hb), hematocrit (HCT), as well as erythrocyte sedimentation rate (ESR), were measured in

blood EDTA-containing tubes. The biochemical analysis was carried out on the plasma/serum and homogenized samples. The serum was used to determine urea, creatinine, total protein, calcium, and liver marker enzymes like aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) [23] [26].

2.6.4 Histopathological analysis

Representative sections' proximal interphalangeal joints per group were separated and immersed in formaldehyde 10% for 24 hours before being immersed in five percent of formic acid. The tissues would then be prepared, fragmented, and paraffin-embedded. The fragments (5µm) were stained with Haematoxylin - eosin and examined under a light microscope (20X intensification) to determine the occurrence of inflammatory cells and the degree of joint damage [12].

2.6.5 Measurement of organ indexes

The animals were sacrificed at the end of the experiment, then the heart, kidney, liver, brain, thymus, and spleen were carefully separated. Immediately after dissection, the rat organs were weighed and observed for any gross aberrations. The following formula was used to determine the organ indexes [24]:

Organ index (%) = [Weight of organ (g)/Bodyweight (g)] X100

2.6.6 Statistical analysis and presentation of data

The values were presented as means and the standardized error of the mean (SEM) using Statistical Package for the Social Sciences (SPSS) Version 22 software. The one-way analysis of variance (ANOVA) followed by Turkey HSD contrast test was used to analyze the data statistical significance. When $p< 0.05$, the differences in measures were deemed important, as well as when $p< 0.01$, the differences in measures were regarded as extremely important [25].

3. RESULTS

3.1 Protective Effects on bodyweight (BW) in CFA-arthritic rats

In this study, we found a link between how severe the joint inflammation was and how much weight the animals lost. This was clear when we compared the healthy animals to the arthritic control group. Compared with the standard and other treatment groups, the arthritic control rats made a good impression. It was found that the weight of the rats was highest in the case of the standard group at 189.04 ± 8.80 gms. Diclofenac and the methanolic extract at the median dose significantly increased the body weight of the animal as compared to the arthritic control group, which was recorded to be 186.64 ± 7.19 gms at 150 mg/kg, while the low and highest doses moderately increased the body weight during the treatment period. Therefore, the extract did not display a dose-dependent increase in the body weight of the rats. At the end of the experiment, the percentages of body weight gain between groups compared to the arthritic control rats were: 54.88%, 50.48%, 36.11%, 48.82%, and 41.46% for the healthy animals, standard medication, low, intermediate, and highest doses of extract, respectively **(Table 1)**.

Table 1: Protective Effects on bodyweight (BW) in CFA-arthritic rats

	BW (gm)Values/ group

Days	Normal Ctl (10mL/kg)	Arthritic Ctl (10mL/kg)	Diclofenac (1.5mg/kg)	Extract 75mg/kg	Extract 150mg/kg	Extract 300mg/kg
0	137.22±3.00	148.20 ± 9.15	151.52 ± 7.01	133.50 ± 2.10	150.34 ± 7.47	144.20 ± 12.25
7	150.94±4.73	158.54 ± 11.04	148.66 ± 5.91	150.08 ± 5.71	162.90 ± 6.15	147.98 ± 13.53
14	164.02±5.62	157.50 ± 9.84	165.24 ± 7.91	154.20 ± 8.17	175.66 ± 5.16	159.56 ± 14.96
21	178.40±5.03	166.78 ± 10.51	189.04 ± 8.80	162.58 ± 9.94	186.64 ± 7.19	175.94 ± 14.40
Changes In BW (Day21-day0)	41.18±2.50 54.88%	18.58 ± 4.69$_{a}^{\cdot}$	37.52 ± 2.69 50.48%	29.08 ± 7.99 36.11%	36.30 ± 3.74 48.82%	31.74 ± 6.25 41.46%

The data represents the mean (n=5) and standard error of the mean (SEM). They were analyzed using one-way ANOVA, followed by Tukey's multiple comparison test. $p > 0.05$ following a similar statistical approach, compared with the arthritic control animals, was **not significant** for all groups. Key: **a**= other groups vs. group 1; ▪ -p<0.001. The percentage values represent the bodyweight gain of groups compared to arthritic control animals. BW: bodyweight; Ctl: Control.

3.2 Protective Effects on paw size in CFA-arthritic rats

There was a significant rise in rat paw diameter in CFA injected control animals when compared to normal rats. After 21 days, it was found that the methanolic extract did not significantly show dose-dependent inhibition in paw thickness. Standard diclofenac sodium significantly decreased the paw thickness changes at 57.43% after induction of Freund's adjuvant, whereas the extract at an intermediate dose significantly decreased the paw thickness at an almost similar level. It was found that in the case of the high dose of the methanolic extract, the percent protection against an increase in paw diameter was found to be 48.14% as compared to that of the low dose, which was found to be 47.70% **(Table 2).**

Table 2: Protective effects on paw size in CFA-injected rats

Days	Paw diameter means/Group					
	Normal Ctl (10mL/kg)	**A. Ctl (10mL/kg)**	**Diclofenac (1.5mg/kg)**	**Extract (75mg/kg)**	**Extract (150mg/kg)**	**Extract (300mg/kg)**
0	2.92±0.07	3.52±0.11	3.52±0.08	3.31±0.04	3.33±0.13	3.29±0.10
1	3.09±0.04	7.69± 0.11	7.16 ± 0.20	7.13 ± 0.15	7.15 ± 0.20	7.37± 0.17
4	3.21±0.05	8.33± 0.16	7.33 ± 0.17	8.32 ± 0.23	8.01± 0.56	7.83± 0.24
7	3.40±0.09	8.44 ± 0.16	7.31 ± 0.19	7.79 ± 0.19	7.24 ± 0.32	7.44 ± 0.35
14	3.63±0.12	8.33± 0.10	6.88± 0.27	6.97± 0.15	6.53± 0.40	6.91± 0.17
21	3.73±0.11	8.90±0.20	5.81±0.16	6.07±0.15	5.61±0.30	6.08±0.16
Changes (Day21-0) (% inh)	0.81±0.13	5.38± 0.17$_{a}^{\cdot}{}_{b}^{\cdot}$	2.29± 0.16$_{a}^{\cdot}{}_{b}^{\cdot}$ (57.43%)	2.76± 0.17$_{a}^{\cdot}{}_{b}^{\cdot}$ (47.70%)	2.28± 0.17$_{a}^{\cdot}{}_{b}^{\cdot}$ (57.62%)	2.79± 0.09$_{a}^{\cdot}{}_{b}^{\cdot}$ (48.14%)

The data represents the mean (n=5) and standard error of the mean (SEM). They were analyzed using one-way ANOVA, followed by Tukey's multiple comparison test. Key: **a**= other groups vs. group 1, and **b=** other groups vs. group 2; **α**-p< 0.05, **Ɛ**-p<0.01, ▪ -p<0.001. The percentage values represent the paw size inhibition of groups compared to arthritic control animals. Ctl: Control.

3.3 Protective Effects on clinical scoring approach in CFA-arthritic rats

During the course of the experiment, the rear paws of all of the different groups of animals began to exhibit symptoms of clinical inflammation. At first, the disease showed up as redness in the joints of the ankle, then, it spread to the joints of the metatarsals and the spaces between the toes. As treatment progressed, the indications of inflammation became more prominent up to day 4. However, there was a brief period of time during which the symptoms of inflammation reduced from the fourth to the tenth day. As shown in **Table 3**, treatment with the standard drug and the intermediate dose of the extract led to the same significant clinical scoring level, while treatment with the low dose or the high dose of the extract led to only a moderate improvement in clinical scores compared to the control groups. The scoring method adopted was previously described by Prodyut et al. [24].

Table 3: Protective Effects on clinical scoring approach in CFA-arthritic rats

Day/ Rats	Values/Group					
	Normal Ctl (10mL/kg)	**Arthritic Ctl (10mL/kg)**	**Diclofenac (1.5mg/kg)**	**Extract (75mg/kg)**	**Extract (150mg/kg)**	**Extract (300mg/kg)**
22/(n=5)	0.00±0.00	$4.00 \pm 0.20_a^{\cdot}$	$1.00 \pm 0.32_b^{\cdot}$	$2.00 \pm 0.51_b^{\varepsilon}$	$1.00 \pm 0.37_b^{\varepsilon}$	$2.00 \pm 0.37_b^{\varepsilon}$

The data represents the mean and standard error of the mean (SEM). They were analyzed using one-way ANOVA, followed by Tukey's multiple comparison test. Key: **a**= other groups vs. group 1, and **b=** other groups vs. group 2; **ε**-p<0.01, **·** -p<0.001.

3.4 Protective Effects on haematological markers in CFA-arthritic rats

The arthritic diseased group increased significantly the concentrations of WBC, PLT, and ESR, and it decreased significantly the concentrations of RBC, Hb, and HCT. Meanwhile, the group administered an intermediate dose was able to return these parameters to the baseline as there was no significant difference between the 150mg/kg treated group and the normal control. A surprising reduction in ESR was seen with all therapies, including diclofenac **(Table 4).** An ESR estimate could be used to determine whether or not a person is experiencing inflammation. In this test, high sedimentation rates are most often linked to RA and other inflammatory conditions. On day 21, ESR was higher in the arthritic control group (10 ± 0.45 mm/h) and lowest in the treated group at 75mg/kg (7.00 ± 0.45 mm/h), with values (mm/h) in Groups 2-4-5 being 4.86 ± 0.39, 5.60 ± 0.51, and 6.60 ± 0.40, respectively.

Table 4: Protective Effects on haematological markers in CFA-arthritic rats

Tests at 22day	Values/group					
	Normal Ctl (10mL/kg)	**Arthritic Ctl (10mL/kg)**	**Diclofenac (1.5mg/kg)**	**Extract (75mg/kg)**	**Extract (150mg/kg)**	**Extract (300mg/kg)**
WBC ($x10^9$/L)	6.90±0.71	$12.84 \pm 1.05_a^{\varepsilon}$	$8.36 \pm 0.99_b^{\alpha}$	10.90 ± 0.77	9.18± 1.01	9.30 ± 1.66
RBC ($x10^{12}$/L)	8.46±0.16	$6.22 \pm 0.37_a^{\alpha}$	7.90 ± 0.34	7.56 ± 0.34	$8.19 \pm 0.20_b^{\alpha}$	7.63 ± 0.16
Hb (g/dL)	16.94±0.24	$12.28 \pm 0.78_a^{\alpha}$	14.72 ± 0.62	13.48 ± 0.65	$16.32 \pm 0.48_b^{\varepsilon}$	14.30 ± 0.37
HCT (%)	50.34±0.65	$36.54 \pm 2.23_a^{\alpha}$	42.60 ± 1.72	38.90 ± 2.11	$48.16 \pm 1.4_b^{\alpha}$	41.22 ± 1.06
PLT ($x10^9$/L)	627±37.93	$1003 \pm 55.77_a^{\alpha}$	778 ± 97.92	$928 \pm 56.28_a^{\alpha}$	732.20± 63.86	$952 \pm 57.32_a^{\varepsilon}$
ESR (mm/hr)	5.60±0.24	$10.00 \pm 0.45_a^{\varepsilon}$	$4.86 \pm 0.39_b^{\varepsilon}$	$7.00 \pm 0.45_b^{\cdot}$	$5.60 \pm 0.51_b^{\cdot}$	$6.60 \pm 0.40_b^{\cdot}$

The data represents the mean (n=5) and standard error of the mean (SEM). They were analyzed using one-way ANOVA, followed by Tukey's multiple comparison test. Key: **a**= other groups vs. group 1, and **b=** other groups vs. group 2; **α-**p< 0.05, **ε**-p<0.01, **·** -p<0.001.

3.5 Protective Effects on biochemical markers in CFA-arthritic rats

The arthritic diseased group increased significantly the concentrations of urea, creatinine, ALT, and AST; it decreased significantly the concentrations of total protein and calcium. Meanwhile, the group administered the extracts was able to return these parameters to the baseline as there was no significant difference between the treated groups and the normal control. This showed that the methanol leaf extract of *E. tomentosa* was effective at treating arthritis in biochemical markers under investigation (**Table 5**).

Table 5: Protective Effects on biochemical markers in CFA-arthritic rats

Tests at 22day	Values/group					
	Normal Ctl (10mL/kg)	**Arthritic Ctl (10mL/kg)**	**Diclofenac (1.5mg/kg)**	**Extract (75mg/kg)**	**Extract (150mg/kg)**	**Extract (300mg/kg)**
UREA (mg/dL)	35.71±2.15	$50.69 \pm 3.21_a^{\varepsilon}$	$28.28 \pm 3.80_b^{\varepsilon}$	$32.07 \pm 3.95_b^{\alpha}$	$32.41 \pm 3.47_b^{\alpha}$	37.93± 2.99
CRT (mg/dL)	1.19±0.07	$2.87 \pm 0.31_a^{\cdot}$	$1.04 \pm 0.07_b^{\cdot}$	$1.23 \pm 0.09_b^{\cdot}$	$1.26 \pm 0.14_b^{\cdot}$	$1.40\pm 0.08_b^{\cdot}$
T. protein (g/dL)	7.35±0.07	$4.06 \pm 0.23_a^{\varepsilon}$	$7.10 \pm 0.23_b^{\cdot}$	$5.12 \pm 0.13_a^{\alpha}{}_b^{\cdot}$	$6.66 \pm 0.18_b^{\varepsilon}$	$6.32\pm 0.15_b^{\varepsilon}$
Calcium (mg/dL)	10.37±0.28	$6.34 \pm 0.61_a^{\cdot}$	$10.09 \pm 0.45_b^{\cdot}$	$8.74 \pm 0.16_b^{\alpha}$	$10.22 \pm 0.65_b^{\cdot}$	$9.53\pm 0.31_b^{\cdot}$
ALT (U/L)	18.22±6.88	$58.78 \pm 10.25_a^{\varepsilon}$	$26.68 \pm 4.53_b^{\alpha}$	$21.74 \pm 4.32_b^{\varepsilon}$	$24.62 \pm 3.95_b^{\varepsilon}$	$19.8\pm5.24_b^{\varepsilon}$
ALP (U/L)	76.54±10.89	56.08 ± 16.03	48.70 ± 5.71	49.68 ± 4.63	46.40 ± 5.28	51.78±11.17
AST (U/L)	15.63±3.22	$56.46 \pm 3.49_a^{\cdot}$	$30.74 \pm 2.79_a^{\alpha}{}_b^{\cdot}$	$26.96 \pm 3.33_b^{\cdot}$	$27.68 \pm 3.79_b^{\cdot}$	$27.7\pm 2.82_b^{\cdot}$

The data represents the mean (n=5) and standard error of the mean (SEM). They were analyzed using one-way ANOVA, followed by Tukey's multiple comparison test. Key: **a**= other groups vs. group 1, and **b=** other groups vs. group 2; **α-***p* < 0.05, **ε**-*p*<0.01, **▪ -***p*<0.001.

3.6 Protective Effects on organ indexes in CFA-arthritic rats

There is a significant increase in all the organ weights across all the groups induced by arthritis. Meanwhile, the group treated with 150mg/kg of the extract was able to reduce the organs' weight to the baseline **(Table 6).**

Table 6: Protective Effects on organ indexes in CFA-arthritic rats

Body organs	Organ indexes (OI) /group					
	Normal Ctl (10mL/kg)	**Arthritic Ctl (10mL/kg)**	**Positive Ctl (1.5mg/kg)**	**Extract 75mg/kg**	**Extract 150mg/kg**	**Extract 300mg/kg**
Heart	0.37±0.01	0.45 ± 0.03	0.41 ± 0.02	0.43 ± 0.04	$0.30 \pm 0.03_b^{\alpha}$	0.44± 0.03
Liver	3.05±0.16	$4.18 \pm 0.28_a^{\alpha}$	3.42 ± 0.11	3.41 ± 0.31	$3.06 \pm 0.17_b^{\alpha}$	$3.17\pm 0.16_b^{\alpha}$
Kidneys	0.59±0.02	$0.83 \pm 0.06_a^{\alpha}$	0.75 ± 0.05	0.79 ± 0.08	$0.63 \pm 0.03_b^{\varepsilon}$	0.76± 0.03
Brain	0.66±0.01	$1.03 \pm 0.06_a^{\varepsilon}$	0.89 ± 0.04	1.01 ± 0.09	$0.78 \pm 0.03_b^{\alpha}$	$0.91\pm 0.05_a^{\alpha}$
Spleen	0.32±0.01	0.39 ± 0.05	0.47 ± 0.01	0.43 ± 0.06	0.38 ± 0.03	0.41± 0.04
Thymus	0.09±0.01	$0.22 \pm 0.02_a^{\cdot}$	0.14 ± 0.02	0.14 ± 0.02	$0.11 \pm 0.01_b^{\varepsilon}$	$0.17\pm 0.01_a^{\alpha}$

The data represents the mean (n=5) and standard error of the mean (SEM). They were analyzed using one-way ANOVA, followed by Tukey's multiple comparison test. Key: **a**= other groups vs. group 1, and **b=** other groups vs. group 2; **α-***p* < 0.05, **ε**-*p*<0.01, **▪ -***p*<0.001.

3.7 Joint histopathological investigation

Generally, the histopathological examinations revealed normal joint morphology for normal control without CFA-induction and no treatment (**Figure 1A**). Rats given the standard medication of diclofenac sodium had mild lymphocytic chronic inflammation in their joints (**Figure 1C**), but no other damage to other cells or tissues. The lowest to the highest dosages of 75, 150, and 300 mg/kg of plant extract presented moderate chronic lymphocytic inflammation without any other lesions on cells or tissues present (**Figure 1D, 1E, and 1F**). Contradictory, the micrographs of the control animals with arthritis showed mixed and severe chronic inflammation, including lymphocytes, neutrophils, giant cells, and tissue necrosis. In short, the general shape of rat joints has almost gone away in negative control animals with arthritis (**Figure 1B**).

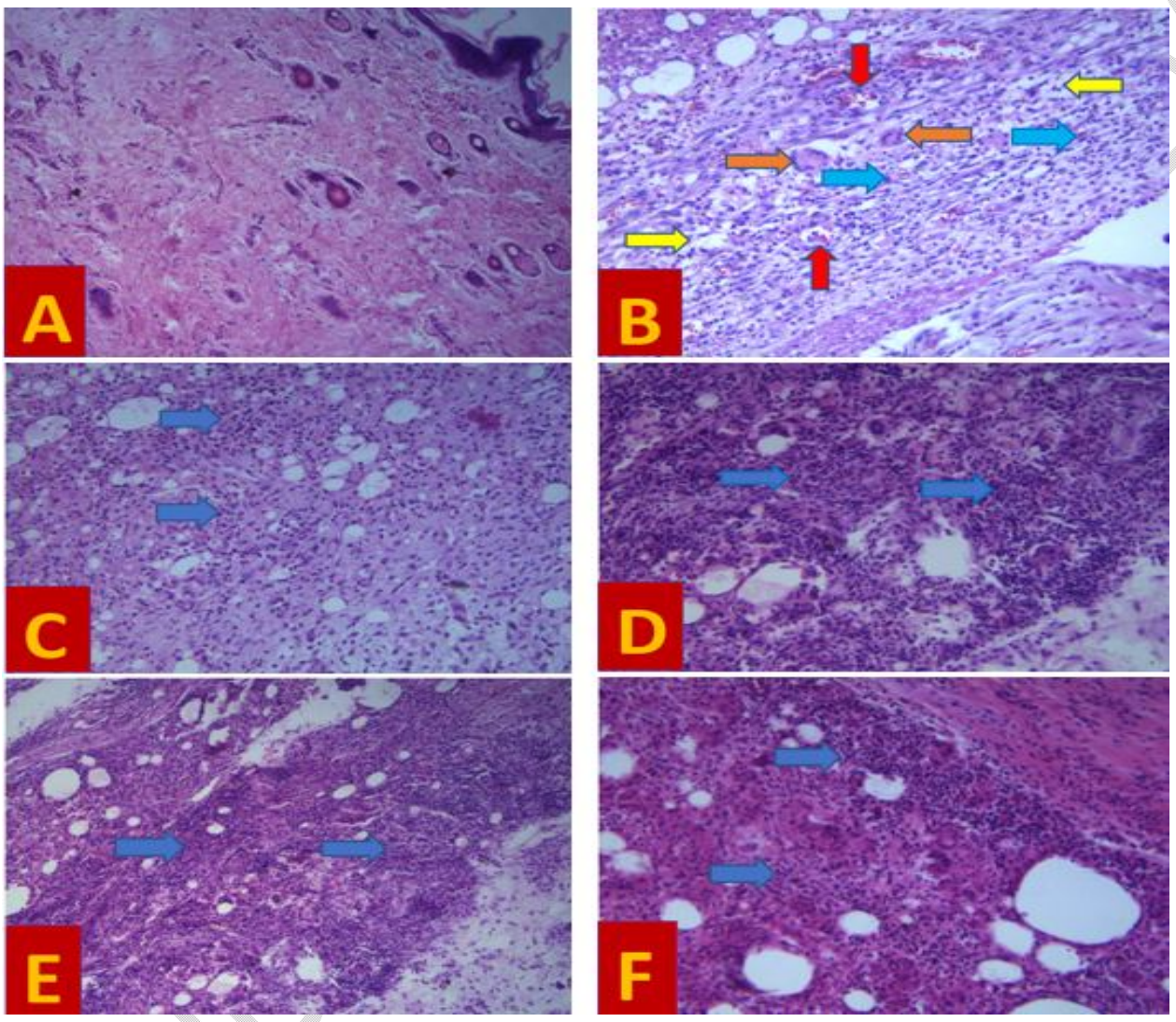

Figure 1: Protective Joint histopathological Effects

A was the normal control group without CFA-induction with no treatment; **B** was the arthritic control group, and **C** was the 1.5 mg/kg diclofenac group.
The doses for groups 4–6 were 75mg/kg, 150mg/kg, and 300mg/kg and were represented by **D**, **E**, and **F**, respectively. The **blue arrow** shows inflammation in the lymphocytes; the **orange arrow** shows inflammation in the giant cells; the **red arrow** shows inflammation in the neutrophil cells; and the **yellow arrow** shows tissue death.

4 DISCUSSION

Rheumatoid arthritis (RA), which is also known as an autoimmune disease, is the most common type of arthritis. The exact cause of RA is not fully understood [27]. Locomotor dysfunction and joint degeneration are all caused by inflammation-mediating substances such as interleukin-1β, 6 and 8 (IL-1β, 6, and 8), tumor necrosis factor-α, interferons

(IFN), and mitogenic growth factor (MGF), the latter discovered to be particularly prevalent in platelets [27–28]. Researchers so far agree that the arthritis model made with Complete Freund's adjuvant (CFA) is the most accurate way to study long-term joint inflammation like human RA in an experimental setting [29]. Rats were chosen because CFA-arthritis induction could cause long-term inflammation in several joints, systemic effects, impairment of joint tendons and cartilage, as well as bone loss, similarly to what happens in human arthritis [30].

In some experiments, the size of the paw is measured by its diameter, while in others, it is measured by its volume. This is an important factor in figuring out how extensive the inflammation is and how well a treatment is effective [31]. In this study, we found that CFA-induced arthritis caused a significant rise in the size of the paws. But therapy with the standard drug and the plant extracts at 75mg/kg, 150mg/kg, and 300mg/kg significantly reduced the paw inflammation by 57.43%, 47.70%, 57.62%, and 48.14%, respectively, compared to the arthritic control rats. This finding is similar to that of Syahirah et al. [31], who reported that 300mg/kg of the aqueous extract of *Centella asiatica* leaves was able to significantly inhibit rats' paw inflammation due to CFA, with 54.19% inhibition.

However, the methanolic extract of *Arisaema propinquum* Schott Rhizomes that Ahad and his colleagues in India studied showed that doses of 200 mg/kg and 400 mg/kg inhibited the paws of the test animals by 63.30% and 80.17 %, respectively [32]. As a result, the activity in our situation was significantly lower. It is interesting to note that our results are much more important than the percentage of inhibition on *Strychnos potatorum* at 200 mg/kg, where aqueous leaf extract showed 35.21%, according to a study done by Ekambaram et al. [33], in India. Particularly interesting is that the *E. tomentosa* leaf extract had the most similar effect on the size of the paws to 1.5 mg/kg of diclofenac**.**These findings demonstrated the extract's ability to significantly reduce arthritic rats' immune system mediation and further delayed the illness's development and lessened its severity.

Other possible arthritis tests, such as hematological and biochemical markers, body weight gain, and changes in organ weight were also evaluated. Body weight changes in animals are considered as signs of diseases states, and it also confirms how the treatment is effective. This is one of the antiarthritis indications that a new molecule is being assessed [30]. In the standard diclofenac therapy and extract-treated groups, there was restoration of the body weights of the treated rats compared to the arthritic control animals, where the percentages recovery of body weights was seen to be 50.48, 36.11, 48.82, and 41.46% for the standard drug; 75, 150, and 300mg/kg of plant extract, respectively. Our results are similar to the findings of Chandel et al. [22], who reported that 250 mg/kg each of aqueous and ethanolic leaf extracts of *Luffa echinata* significantly increased rats' body weight by 30.53% and 36.75%, respectively. Also, these results are similar to the report of Srivastava et al. [29], who reported percentage of body weight gain of 23.03% for 400mg/kg and 35.00% for 800mg/kg methanolic extract aerial parts of *Costus speciosus*, respectively, in rats.

Scientists reported that when the body is inflamed, nutrients aren't well absorbed through the intestinal tract, which makes the body to lose weight. Then antiinflammatory therapy reduces inflammation, increases absorption, which enhance weight increase [27]. Dietary absorption and a lessened impact of arthritic pain may be responsible for the animals in the extract and diclofenac therapy subjects regaining their pre-arthritis weight, in which animals on the test and standard medications lost weight more slowly than those on the controls.

When a person's immune system responds to interleukin-1 by making more glycoproteins that control how granulocytes work, they have a high number of white blood cells (WBC), like people with arthritis [34]. The results so far show that treated animals are more likely to keep their WBC count at normal levels. Other alterations that could happen to the blood cells include a lower Hb count and a higher ESR. Both of these changes were also maintained by the extract, at 150 mg/kg, while diclofenac and other extract doses were able to lower the ESR**.** Studies suggest that a drop in Hb levels is caused by a weaker response from the bone marrow and early damage to erythrocytes, because erythropoietin levels are going down [27–32]. In a similar way, a rise in the ESR is a sign of an ongoing illness that hasn't been identified yet, because it's linked to an escalation in the production of endogenic proteins, like alpha or beta globulin as well as fibrinogen [34]. Therefore, the fact that extract therapy lowers ESR while raising Hb levels suggests that it may help people with arthritis. The most effective dose of the plant extract was found to be 150mg/kg**.**

Furthermore, checking the levels of serum ALP, AST, and ALT, as well as bilirubin, is a good and easy way to figure out how well the medicine of interest helps with arthritis [35]. Blood enzymes like ALP, ALT, and AST, as well as other markers like bilirubin, can show that the liver is not working as well as it should [36]. Based on the study findings, the fact that these serum enzymes were very high shows that the effect was bad for the control rats with arthritis**.** High levels of

AST, ALT, and ALP, as well as creatinine and urea, and low levels of protein, and calcium in control rats with arthritis show that their livers, kidneys, and spleens are getting damaged. This situation is also a part of the adjuvant arthritis model in rats, and it was recovered in all test groups, including the standard drug test group [35–36].

The fact that the levels of AST and ALT enzymes reduced in rats that had been given a plant extract, showed that *E. tomentosa* extract could help people with arthritis. This was shown by a decrease in bone loss and an increase in the extract's ability to protect organs. Thymus and liver growth have been shown to be caused by immune system stimulation [34]. The fact that the extract-treated animals' thymus and liver indices decreased, shows that many kinds of cells in these tissues have been altered, indicating adaptations in immunological effect. Note that antirheumatic treatments slow the growth of new inflammatory cells, which is why the indices in the spleen, liver, and thymus decreased [27]. The plant extracts, specifically 150mg/kg, also displayed similar effects on these organs. Even though these results demonstrate that the plant extract has immunosuppressive potential, this activity needs to be validated directly in order to be true.

Histopathological tests showed that the normal morphology of the joint had broken down significantly more for arthritic rats than in treated animals at the end of the experiment. The results showed that both the plant extracts and the standard therapy helped keep the cells in the animals' tissues from dying**.**

Alkaloids, terpenoids, coumarins, tannins, glycosides, flavonoids, saponins, and phenols were found in the plant in the preliminary phytochemical analysis. There are many compounds, such as saponins, alkaloids, tannins, and flavonoids that are known to help with inflammation and arthritis [37]. So, the activity that was shown could be linked to some of the phytoconstituents that were found in the methanol leaf extract of the plant. More research needs to be done to learn about the plant's different biological functions and how the molecules that cause these effects work.

5Conclusion

The present overall data revealed the beneficial antiarthritic effect of all plant extract doses of *E. tomentosa* leaf extract and was more specific at the dose level of 150 mg/kg in the experimental CFA-induced arthritis rat model of both physical and systemic antiarthritic tests. This study found that the effect on arthritis was not dose-dependent, since 300mg/kg was a bit less effective than the median dose of 150mg/kg. In addition, treated groups including standard drugs showed a reduction in rat paw edema diameter and they could normalize the haematological and biochemical abnormalities in adjuvant induced arthritic rats in both the evolving and developed phases of CFA induced arthritis. The histopathological studies confirmed the antiarthritic activity of the plant extract in CFA-induced arthritic rats. The actual mechanism of action of plant extract on adjuvant-induced arthritic rats is still unclear with these studies. Researchers should examine how *E. tomentosa* methanol leaf extract affects the specific mediators of inflammation such as interleukins and tumor necrosis factor-α (TNF-α), among others. Also, studies must be done to discover and isolate the active chemicals that produce the activity.

CONSENT

It was not applicable.

ETHICAL APPROVAL

The Animal Research Ethical Committee of KIU-WC (AREC/KIU-WC) approved all procedures under the KIU-2022-86 number before experiments. Additionally, the Guidelines for the Uganda National Health Laboratory Hub and Sample Transport Network, published in 2017, and the National Academies Press's Guide for the Concern and Usage of Laboratory-Animals, published in Washington, DC, were followed to ensure animal health and rights (19–20).

REFERENCES

1. Hajja G, Bahlouli, A. Medicinal plants in the prevention and treatment of rheumatoid arthritis. MOJ Bioequivalence & Bioavailability 2018, 5 (1). DOI: 10.15406/mojbb.2018.05.00084.
2. Sankar M. Arthritis: Classification, Nature & Cause -A Review. American Journal of Bio-Pharmacology Biochemistry and Life Sciences. 2013; 02(03): 01-19.
3. Arend WP. Arthritis & Rheumatism. Official Journal of the American College of Rheumatology.1997; 40(4): 595-597

4. Usenbo A, Kramer V, Young T, Musekiwa A. Prevalence of Arthritis in Africa: A Systematic Review and Meta-Analysis. PLoS One 2015; 10 (8), e0133858. DOI: 10.1371/journal.pone.0133858
5. Ding Q, Hu W, Wang R, Yang Q, Zhu M, Li M, Cai J, Rose P, Mao J, Zhu YZ. Signaling pathways in rheumatoid arthritis: implications for targeted therapy. Signal Transduction and Targeted Therap. 2023; 8 (1), 68. DOI: 10.1038/s41392-023-01331-9.
6. Yoshiya T. Rheumatoid arthritis: Review. BMC.2020; 40(20): 1-8.
7. Yvette C, Terrie BS. 2021. Challenges in managing and preventing rheumatoid arthritis pain. Rheumatology Research Foundation. 2021; 45(3): 42-47.
8. Donahue KE, Jonas DE, Hansen RA, Roubey R, Jonas B, Lux LJ, Gartlenher G, et al. Drug therapy for rheumatoid arthritis in adults. Comparative Effectiveness Review. 2012; 55(8): 1-1073.
9. Arthritis F. Arthritis by the Numbers. Arthritis Foundation. 2019; 3(17): 1-102
10. Roy T, Ghosh S. Animal Models of rheumatoid arthritis: Correlation and usefulness with human rheumatoid arthritis. Indo American Journal of Pharmaceutical Research. 2013; 3(8): 6131-6142.
11. Hassan R, Faruqui H, Alquraa R, Eissa A, Alshaiki F, Cheikh, M. Classification Criteria and Clinical Practice Guidelines for Rheumatic Diseases. In Skills in Rheumatology, Almoallim, H., Cheikh, M. Eds. 2021; pp 521-566.
12. Cui X, Wang R, Bian P, Wu Q, Seshadri VDD, Liu L. Evaluation of antiarthritic activity of nimbolide against Freund's adjuvant induced arthritis in rats. Artif Cells NanomedBiotechnol 2019; 47 (1), 3391-3398. DOI: 10.1080/21691401. 2019.1649269
13. Ssenku JE, Okurut S A, Namuli A, Kudamba A, Tugume P, Matovu P, et al. Medicinal plant use, conservation, and the associated traditional knowledge in rural communities in Eastern Uganda. Trop Med Health. 2022; 50 (1), 39. DOI: 10.1186/s41182-022-00428-1
14. Patience T, Clement N. Ethno-pharmacological survey of herbal remedies used in the treatment of pediatric diseases in Buhunga parish, Rukungiri District, Uganda. BMC Complementary and Alternative Medicine. 2019; 19(1): 1-10.
15. Isaac M, Samuel BO, Ivan I, Pender GC, Adam MA & Saidi O. Antinociceptive and anti-inflammatory activities of the aqueous leaf extract of *Erlangea tomentosa* (Asteraceae) in rats and mice. Journal of Pharmacy & Bioresources. 2020; 17(1):19-23.
16. Berhan B, Bharat M, Asegedech T, Zewdneh S. Methanol leaves extract Hibiscus micranthus Linn exhibited antibacterial and wound healing activities. BMC Complementary and Alternative Medicine. 2017; 17: 1-11.
18. Hawkins P, Rachel A, Tania B, Paul G, Katherine K, Elliot L, et al. Applying refinement to the use of mice and rats in rheumatoid arthritis research. Inflammopharmacology. 2015; 23: 131-150.
19. Uganda MH. Guidelines for the Uganda National Health Laboratory Hub and Sample Transport Network. Ministry of Health. 2017; 1:1-36
20. Liu Y, Huang Y, Xiao Z, Ren X, Yang C. Guide laboratory for the care and use of animals. In Gongcheng/Materials Science and Engineering of Powder Metallurgy. 2016; 21:1-12.
21. Vendors AA, Conditions BH, Strains CR. A Protocol for Adjuvant-Induced Arthritis (AIA) in Rats. Chondrex, Inc. 2020; 1-4.
22. Chandel H, Singh S, Kushwaha R. Evaluation of antiarthritic activity on *Luffa echinata* Roxb. Fruits on rats. Asian Journal of Biomedical and Pharmaceutical Sciences. 2013; 3(21): 36-41.
23. Manimekalai P, Shukla KMP, Sudhakar P, Murugananthan G. Synthesis and evaluation of phthalate analogue of diclofenac against Freund's Complete Adjuvant induced arthritis in rat. International Journal of Pharmacy and Pharmaceutical Sciences. 2019; 11(12): 1-6
24. Mondal P, Sonjit D, Kabita M, Sudarshana B, Ali JJ, Zaman K. Evaluation of anti-arthritic potential of the hydro-alcoholic extract of the stem bark of *Plumeria rubra* in Freund's complete adjuvant-induced arthritis in rats. Journal of Chemical Information and Modeling. 2016; 7(9): 1689-1699.
25. Soni H, Patel G, Shah M, Panchal M, Murti K. Evaluation of anti-arthritic activity of Dazzle ointment-A polyherbal formulation. International Journal of Pharmacology and Clinical Sciences. 2013; 2(1): 14-18.
26. Mbiantcha M, Almas J, Shabana SU, Nida D, Aisha F. Anti-arthritic property of crude extracts of *Piptadeniastrumafricana* (Mimosaceae) in complete Freund's adjuvant-induced arthritis in rats. BMC Complementary and Alternative Medicine. 2017; 17(1): 1-16.
27. Majid M, Nasir B, Zahra SS, Khan MR, Mirza B, Haq I. *Ipomoea batatas* L. Lam. ameliorates acute and chronic inflammations by suppressing inflammatory mediators, a comprehensive exploration using in vitro and in vivo models. BMC Complementary and Alternative Medicine.2018; 18(1): 1-21.

28. Bakshi AV, Begum NVK, Kumar NY, Reddy Y. Anti-arthritic and anti-inflammatory activity of *Beta Caryophyllene* against Freund's Complete Adjuvant-induced arthritis in Wistar rats. Journal of Bone Reports & Recommendations.2015; 1(9):1-10
29. Srivastava S, Singh P, Jha KK, Mishra G, Srivastava S, Khosa RL. Evaluation of antiarthritic potential of the methanolic extract of the aerial parts of *Costus speciosus*. Journal of Ayurveda and Integrative Medicine.2012; 3(4): 204-208.
30. Pushpan R, Karra N, Nariya MB. Evaluation of anti-arthritic potential of *Leonotis nepetifolia* R.Br. against Freund's adjuvant induced arthritis. Journal of Ayurveda and Integrated Medical Sciences (JAIMS).2017; 2(05): 59-66.
31. Syahirah W, Althunibat OY, Hanis F. Antiarthritic effects of water extraction of *Centella asiatica* L. Leaves on adjuvant-induced arthritis in rats. International Journal of Recent Scientific Research. 2016; 4(11): 1701-1705.
32. Ahad MP, Mohi R, Akbar DM, Bader NG. Annals of pharmacology and pharmaceutics evaluation of anti-arthritic potential of *Arisaema propinquum* Schott Rhizomes against Complete Freund's adjuvant Induced arthritic rats. Remedy Publications LLC. 2020; 5(2): 1-8.
33. Ekambaram S, Perumal SS, Subramanian V. Evaluation of antiarthritic activity of *Strychnos potatorum* Linn seeds in Freund's adjuvant induced arthritic rat model. BMC Complementary and Alternative Medicine. 2010; 10(56): 1-9.
34. Patil KR, Jadhav RB, Mahajan VK, Patil PR, Gaikwad PS. Anti-arthritic activity of bartogenic acid isolated from fruits of *Barringtonia racemosa* Roxb. (Lecythidaceae). Evidence-Based Complementary and Alternative Medicine. 2011: 1-7.
35. Abdul M, Kadhem H. Anti-arthritic activity of ethanolic extract of *Lawsonia inermis* in Freund ' s Adjuvant-induced arthritic rats. IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS).2016; 9(6):1-6
36. Kumari RP, Anbarasu K. Protective Role of C-Phycocyanin against secondary changes during sodium selenite mediated cataractogenesis. Natural Products and Bioprospecting. 2014; 4(2): 81-89.
37. Reis NC, Arantes BM, Menezes FPS, Leandro CL, De Souza PM, Pereira ML, Curcino VIJ, Oliveira D. 2020. Plants as sources of anti-inflammatory agents. Journal of Molecules; 25(3726): 1-22.