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AQUEOUS LEAF EXTRACT OF *Paullinia pinnata* (LINN.) PREVENTS ARTHRITIS AND BOOSTS ANTIOXIDANT ACTIVITIES IN FORMALDEHYDE-INDUCED ARTHRITIC RATS

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ABSTRACT

The study assessed the antiarthritic and antioxidant effects of aqueous extract of Paullinia pinnata leaf (AEPPL) in formaldehyde-induced arthritic rats. A total of thirty female Wistar rats were randomized into six groups (A-F) of five rats. Each group received distinct treatments: Group A served as the normal control and received distilled water, while groups B to F were pre-treated orally with varying doses of AEPPL or diclofenac, a known anti-inflammatory agent. Throughout the nine-day experimental period, parameters including paw thickness and body weight were meticulously monitored. Arthritis induction was done on day 1 and replicated on the third day to ensure sustained inflammatory conditions. Following the treatment regimen, the rats were sacrificed, and analysis carried out on blood and liver of the animals. Remarkably, AEPPL exhibited a significant reduction in paw thickness and alleviated body weight loss, particularly evident at the dosage of 100 mg/kg. Hematological analyses unveiled improvements in key parameters, such as white blood cell count, red blood cell count, and hemoglobin levels, notably in groups treated with 50 and 100 mg/kg AEPPL. Antioxidant enzyme activities, such as superoxide dismutase (SOD), glutathione (GSH), and catalase, exhibited dose-dependent increase in AEPPL-treated rats. Histopathological examinations of the ankle joint corroborated these findings, revealing diminished inflammation in rats treated with AEPPL. These comprehensive results underscore the potential of AEPPL as a promising source for the development of antiarthritic agents, owing to its robust antioxidant properties and notable therapeutic efficacy.

Keywords: *Anti-arthritic, formaldehyde, inflammatory cells, oedema, Paullinia pinnata*

INTRODUCTION

Arthritis, a chronic inflammatory disease that affects the joints, can cause severe disability and a reduced quality of life (Richardson, 2021). It is a leading cause of disability worldwide, impacting millions of people, including Nigerians, especially the elderly (Ekediegwu et al., 2022). There are over 100 conditions that affect the joints, surrounding tissues, and other connective tissues. The most common types are osteoarthritis (OA) and rheumatoid arthritis (RA) (Lindler *et al.,* 2020). RA is a common autoimmune disease associated with progressive disability and systemic complications which when left untreated, can result in the gradual destruction and deformity of the joints, leading to long-term disability, chronic pain, and premature death (Gautam *et al.,* 2021). It is characterized by pain, swelling, and stiffness in the synovial joints (Hossein-Khannazer *et al.,* 2022). As of 2020, approximately 17.6 million people were living with this condition globally and it is expected to rise to 31.7 million by 2050. The prevalence rate of rheumatoid arthritis has increased by 14.1% since 1990, with a female-to-male ratio of 2.45 . This highlights the need for greater awareness and support for those affected by this disease.

Treatment goals for RA include symptom elimination, slowing disease progression, and improving quality of life (Almoallim *et al.,* 2021). However, many medications for arthritis have adverse effects including heartburn, belching, nausea, headache, fatigue (Köhler *et al.,* 2019); and as a result, there is growing interest in exploring alternative treatments, particularly natural products such as plants (Dudics et al., 2018), and one potential alternative is *Paullinia pinnata* (Singh *et al.,* 2020).

Paullinia pinnata (Linn.) is a plant species from the *Sapindaceae* family that is found in tropical regions of South and Central America, as well as subtropical and tropical areas of Africa. It has been used in traditional medicine to treat various diseases, including arthritis (Tseuguem *et al.,* 2019).

The aqueous extract of *Paullinia pinnata* (Linn.) leaf has demonstrated significant anti-inflammatory and analgesic activities in experimental models (Tseuguem *et al.,* 2019). It has also shown antioxidant properties, which can protect against oxidative stress-induced damage, a major contributor to arthritis development (Nafiu *et al.,* 2018). These combined activities make it a potential therapeutic agent for arthritis management (Faisal *et al.,* 2022). Therefore, this study assessed the antiarthritic and antioxidant activities of *Paullinia pinnata* (LINN.) leaf aqueous extract in formaldehyde-induced arthritic rats.

Materials and Methods

Plant Materia

Fresh leaves of *Paullinia pinnata* were obtained from Ijawaya Secondary School premises along Ogbomoso Road, Oyo state, Nigeria. The plant was identified and authenticated at the Herbarium of the National Institute for Pharmaceutical Department of Plant Biology herbarium, Research and Development, Abuja, Nigeria. A specimen with voucher number Herb/NIRPD/6404 was deposited for future reference.

Experimental Animals

Thirty (30) adults female Wistar rats (180 ± 20 g) were obtained from the animal holding unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. All the rats were acclimatized under standard laboratory conditions (housed in clean cages provided with husk bedding and maintained at 24°C ± 2°C under 12 hrs. light/ dark cycle for two (2) weeks. They were fed with standard rodent feed supplied by Top Feed Industries Limited, Ibadan, Nigeria, and had free access to tap water.

Chemicals and Reagents

Diclofenac sodium was a product of Bliss Pharmaceutical Ltd., and Formaldehyde was a product of Guangdong Guanghua Chemical Co., Ltd. (JHD). All other reagents used were of analytical grade and were prepared using distilled water or suitable solvent and stored under appropriate conditions.

Methods

Preparation of Aqueous Extract of *Paullinia pinnata* Leaf (AEPPL)

Fresh leaves of *Paullinia pinnata* were thoroughly rinsed under running water to remove dirt. Thereafter, the leaves were air dried under shade and pulverized into powder. The powdered (500 g) leaf was exhaustively extracted with 3 litres of distilled water within 48 hours. The extract was then filtered using Whatman No. 1 filter paper and concentrated using a water bath at 45°C. The concentrate was kept in an airtight container at 4-8°C in the refrigerator until the time of use for study.

Animal Grouping and Extract Administration

A total of thirty female Wister rats were used in this study. The animals were randomly selected into six groups of five rats each with administration done once daily for nine days as

A: Non-induced rats + 0.5 ml of normal saline.
B: Arthritic rats + 0.5 ml of normal saline.
C: Arthritic rats + 0.5 ml of 10 mg/kg diclofenac sodium.
D: Arthritic rats + 0.5 ml of 25 mg/kg body weight of AEPPL.
E: Arthritic rats + 0.5 ml of 50 mg/kg body weight of AEPPL.
F: Arthritic rats + 0.5 ml of 100 mg/kg body weight of AEPPL.

Induction of Experimental Arthritis, Preparation of serum and Tissue homogenate

Arthritis was induced using the method described by Fatima (2021). In summary, arthritis was induced by injecting 100 µl of 2% formaldehyde (v/v) into the right hind paws of the animals, 30 minutes after administering the extract orally on days 1 and 3. To assess paw thickness, a micrometer screw gauge was used to measure the baseline paw thickness on day 0 of the experiment. The extent of edema in arthritis was determined by calculating the difference in paw thickness between day 0 and days 2, 4, 6, 8, and 10. The animals' body weight was measured using a weighing balance, and hematological parameters were analyzed using a Sysmex automated hematological analyzer. Serum and tissue homogenates were prepared as described by Yusuf *et al.,* .

Biochemical assays and Histopathology

Superoxide dismutase (SOD) was assayed as reported by (Mistra & Fridovich, 1972). Catalase was assayed according to the procedure described by Beers and Sizer (1952). Reduced glutathione (GSH) was measured by the method of Beutler and Kelly (1963). The concentration of MDA was quantified according to the method of Nelson and Nusse (2004), nitric oxide was assayed for as described by Wo *et al.* (2013). Histology of tissues was done as described by Drury and Wallington (1980).

Statistical Analysis

Data were expressed as the mean ± SEM of five determinations. Data were analyzed using one-way analysis of variance followed by Tukey's post-hoc test for multiple comparisons. Statistical significance was set at a 95% confidence interval ($p < 0.05$) and GraphPad Statistical Package version 6.0 was used for the statistical analyses.

Results

There was a continuous increase in paw oedema of arthritic untreated rats throughout the experimental period as compared to other test groups. Significant ($p < 0.05$) increase in paw thickness observed in days 2 and 4 was reversed to the baseline level (day 0) from day 6 in animals treated with 25 mg/kg while the reversal at 50 mg/kg b.wt AEPPL commenced on day 8. At 100 mg/kg, the reversal only occurred on day 8 and could not be sustained (Table 1).

Table 2 shows the body weight of rats before and after experimental induction of arthritis. The body weight of the arthritic untreated animals significantly ($p <0.05$) decreased as compared with the non-induced control group on day 0. Treatment with 100 mg/kg b.wt AEPPL resisted the formaldehyde-induced weight reduction and compared well

with non- induced control group as well as those treated with diclofenac until after day 6.

Table 3 shows the hematological parameters of formaldehyde-induced arthritic rats treated with AEPPL. The WBC, RBC, HGB, HCT, and MCV of the test and reference groups compared favorably with that of the control except for the untreated group and 25 mg/kg b.wt AEPPL, which showed a significant decrease ($p < 0.05$). Also, there was no significant ($p > 0.05$) difference in the values of MCH and MCHC across all groups.

There was a significant ($p>0.05$) increase in the nitric oxide level of the untreated group when compared with the control group (Figure 1). Administration of varied concentrations of AEPPL manifested a significant ($p< 0.05$) reduction in the nitric oxide level except at 25 mg/kg b.wt AEPPL when compared with the control group. A marked reduction seen in the 100mg/kg b.wt AEPPL group shows relative nitric oxide activity with that of the reference (10mg/kg b.wt diclofenac) group.

The activity of superoxide dismutase (SOD) in the untreated arthritic animals showed a significant decrease ($p < 0.05$) compared to the non-induced control group (Figure 2). However, oral administration of 100 mg/kg body weight of AEPPL restored the SOD activity to levels that were not significantly different from the non-induced control group. The hepatic catalase activity in arthritic untreated rats exhibited a significant decrease ($p < 0.05$) compared to the values observed in non-induced control rats (Figure 3). Treatment of arthritic rats with 50 mg/kg body weight and 100 mg/kg body weight of AEPPL resulted in a significant ($p > 0.05$) increase in hepatic catalase levels, bringing the concentration back to values that were not significantly different from those recorded for non-induced control rats.

The study demonstrates that rats with formaldehyde-induced arthritis when treated with AEPPL, exhibited a notable increase in reduced glutathione (GSH) concentrations (figure 4). AEPPL treatment effectively enhanced GSH concentrations, while causing a decrease in malondialdehyde (MDA) concentrations across all groups except the untreated group. The reference-treated animals showed the lowest MDA level concentration. Notably, no significant difference was observed between the AEPP-treated group and the non-induced control group (figure 5).

The bone tissue of the control rat displayed normal synovium (Plate 1). In the arthritic untreated group, the bony tissue showed significant soft tissue swelling, synovial hyperplasia, increased vascularity, and the presence of inflammatory cells (lymphocytes) (Plate 2). Plates 3 and 4, taken from the hind limbs of arthritic rats treated with diclofenac and AEPPL at doses of 10 and 25 mg/kg b.wt., respectively, exhibited signs of synovial membrane re-establishment, reduced edema, and fewer inflammatory cells. Plates 5 and 6, treated with 50 and 100 mg/kg body weight of AEPPL, respectively, showed a marked reduction in edema and inflammatory cells in the bony tissue. Table 1: Effect of aqueous extract of Paullinia pinnata leaf on paw thickness of formaldehyde-induced arthritic rats

Table 1: Effect of aqueous extract of *Paullinia pinnata* leaf on paw thickness of formaldehyde-induced arthritic rats

	Days					
Treatment Groups	0	2	4	6	8	10
Normal control	3.19 ± 0.08^{a}	3.21 ± 0.10^{a}	3.19 ± 0.02^{a}	3.23 ± 0.07^{a}	3.22 ± 0.10^{a}	3.23 ± 0.07^{a}
Arthritic untreated	3.35 ± 0.09^{a}	5.45 ± 0.16^{b}	5.55 ± 0.86^{b}	5.56 ± 0.29^{c}	6.49 ± 0.98^{b}	6.53 ± 0.88^{b}
10 mg/kg b.wt DFNS	3.51 ± 0.37^{a}	3.86 ± 0.19^{a}	4.67 ± 0.12^{b}	4.36 ± 0.12^{b}	3.77 ± 0.41^{a}	3.58 ± 0.09^{a}
25 mg/kg b.wt AEPPL	3.33 ± 0.10^{a}	5.51 ± 0.13^{d}	5.00 ± 0.48^{c}	3.79 ± 0.18^{b}	$3.92 \pm 0.79^{b,c}$	3.87 ± 0.59^{b}
50 mg/kg b.wt AEPPL	3.50 ± 0.08^{a}	5.65 ± 0.14^{c}	4.41 ± 0.42^{b}	$5.10 \pm 0.50^{b,c}$	3.66 ± 0.20^{a}	3.60 ± 0.20^{a}
100 mg/kg b.wt AEPPL	3.46 ± 0.53^{a}	4.81 ± 0.7^{b}	$5.65 \pm 0.63^{b,c}$	$4.11 \pm 0.52^{a,b}$	$3.89 \pm 0.47^{a,b}$	$4.06 \pm 0.38^{a,b}$

Values are mean of 5 replicates ± SEM.
values carrying different superscripts are significantly ($P < 0.05$) different from the control.
Where:
DFNS = Diclofenac Sodium and AEPPL = Aqueous Extract of Paullinia pinnata (LINN) Leaf.

Table 2: Effect of aqueous extract of *Paullinia pinnata* leaf on body weight changes of formaldehyde-induced arthritic rats

	Days					
Treatment Groups	0	2	4	6	8	10
Normal control	195.33 ± 2.33^{a}	189.00 ± 1.00^{b}	188.00 ± 3.06^{b}	187.00 ± 2.08^{b}	187.00 ± 4.04^{b}	186.67 ± 6.74^{b}
Arthritic untreated	196.33 ± 3.18^{a}	179.33 ± 3.53^{b}	179.33 ± 5.70^{b}	184.33 ± 8.18^{b}	184.33 ± 5.24^{b}	178.0 ± 9.64^{b}
10 mg/kg b.wt DFNS	230.33 ± 10.35^{a}	233.33 ± 9.87^{a}	229.67 ± 11.80^{a}	228.33 ± 11.39^{a}	229.33 ± 8.35^{a}	228.00 ± 8.50^{a}
25 mg/kg b.wt AEPPL	209.33 ± 1.33^{a}	198.00 ± 9.54^{b}	201.33 ± 3.48^{b}	193.33 ± 10.09^{b}	189.67 ± 10.40^{b}	178.00 ± 9.64^{b}
50 mg/kg b.wt AEPPL	210.67 ± 3.48^{a}	206.33 ± 4.63^{a}	$200.00 \pm 5.03^{a,b}$	198.67 ± 0.88^{b}	195.67 ± 1.86^{b}	195.67 ± 1.46^{b}
100 mg/kg b.wt AEPPL	222.00 ± 4.62^{a}	214.00 ± 3.21^{a}	206.33 ± 1.67^{b}	211.67 ± 8.01^{a}	205.67 ± 4.18^{b}	204.00 ± 2.65^{b}

Values are mean of 5 replicates ± SEM.
values carrying different superscripts are significantly ($P < 0.05$) different from the control
Where:
DFNS = Diclofenac Sodium and AEPPL = Aqueous Extract of Paullinia pinnata (LINN) Leaf.

Table 3: Effect of aqueous extract of *paullinia pinnata* leaf on Haematological parameters of formaldehyde-induced arthritic rats

Treatment Groups	WBC ($x10^3/\mu L$)	RBC ($x10^3/\mu L$)	HGB (g/dL)	HCT (%)	MCV (fl)	MCH (pg)	MCHC (g/dL)	PLT ($x10^3/\mu L$)	LYM (%)
Normal control	7.73 ± 1.89 [a]	6.00 ± 0.70 [a]	8.90 ± 1.54 [a]	37.80 ± 4.05 [a]	63.35± 3.13 [a]	14.78 ± 1.64 [a]	23.25 ± 2.49 [a]	378.50 ± 87.5 [a]	72.75 ± 9.84 [a]
Arthritic untreated	4.08 ± 1.30 [c]	4.00 ± 0.59 [b]	5.95 ± 0.90 [c]	24.53 ± 2.96 [b]	56.67 ± 0.61 [c]	14.90 ± 0.20 [a]	23.98 ± 0.82 [a]	284.25 ± 60.7 [a]	64.63 ± 0.68 [a]
10 mg/kg b.wt DFNS	11.30 ± 0.41 [b]	7.13 ± 0.46 [a]	11.10 ± 0.26 [b]	40.40 ± 1.06 [a]	62.28 ± 2.06 [a]	15.57 ± 0.20 [a]	27.50 ± 0.06 [a]	833.00 ± 7.72 [c]	87.20 ± 4.41 [b]
25 mg/kg b.wt AEPPL	4.23 ± 1.28 [c]	4.75 ± 0.61 [b]	7.07 ± 1.52 [a]	28.73 ± 4.72 [b]	56.03 ± 0.04 [c]	14.60 ± 1.19 [a]	24.27 ± 1.43 [a]	277.33 ± 49.4 [a]	54.13 ± 10.6 [a]
50 mg/kg b.wt AEPPL	8.95 ± 0.94 [a]	6.98 ± 0.13 [a]	10.73 ± 0.17 [b]	40.88 ± 0.86 [a]	58.55 ± 1.26 [a,b]	15.38 ± 0.37 [a]	26.25 ± 0.25 [a]	658.25 ± 72.0 [b]	62.95 ± 2.36 [a]
100 mg/kg b.wt AEPPL	9.06 ± 1.62 [a]	7.13 ± 0.46 [a]	11.10 ± 0.26 [b]	40.40 ± 1.06 [a]	59.67 ± 0.61 [a]	15.57 ± 0.20 [a]	27.50 ± 0.06 [a]	833.00 ± 7.72 [c]	87.20 ± 4.41 [b]

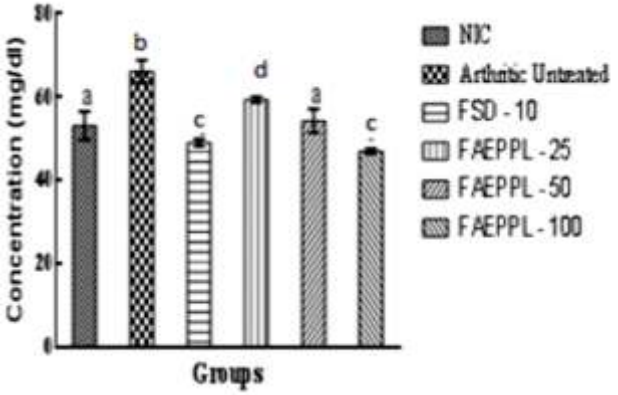

Figure 1: Serum Nitric oxide levels in formaldehyde-induced arthritic rats treated with AEPPL.

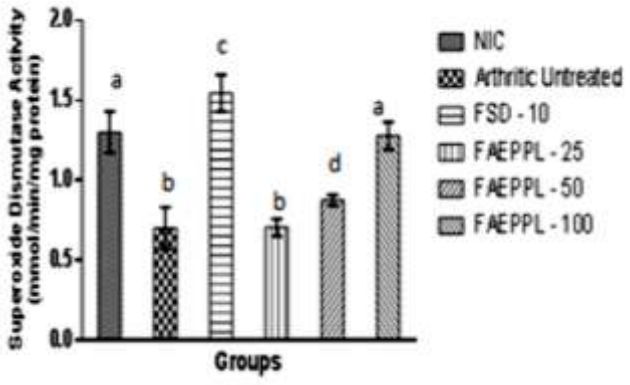

Figure 2: Hepatic Superoxide Dismutase activity in formaldehyde-induced arthritic rats treated with AEPPL.

Figure 3: Hepatic Catalase activity in formaldehyde-induced arthritic rats treated with AEPPL.Bars are expressed as mean of five replicates ±SEM; Bars with different superscripts are significantly different ($p < 0.05$) from the control.

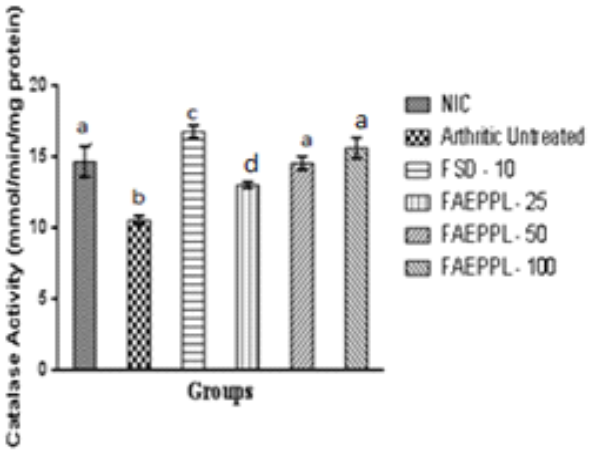

Figure 4: Reduced glutathione concentration in formaldehyde-induced arthritic rats treated with AEPPL.

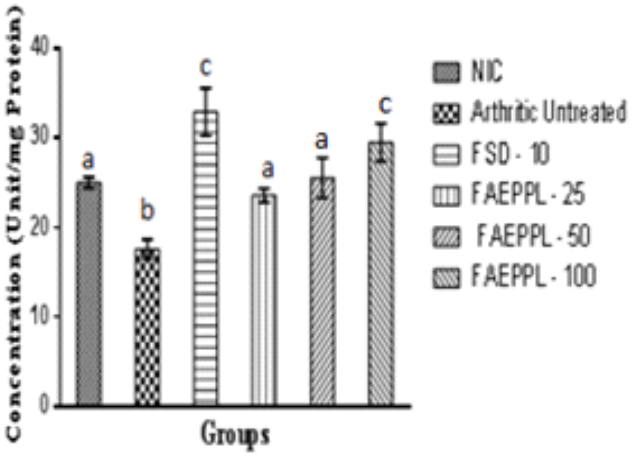

Figure 5: Malondialdehyde concentration in formaldehyde-induced arthritic rats treated with AEPPL.

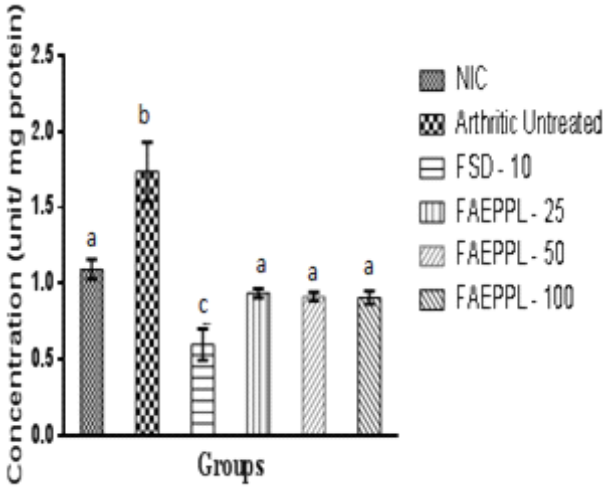

Plate 1: Photomicrograph (X400) of Normal Rat hind limb treated with distilled water.
The bony tissue showed normal cartilage and normal synovium.

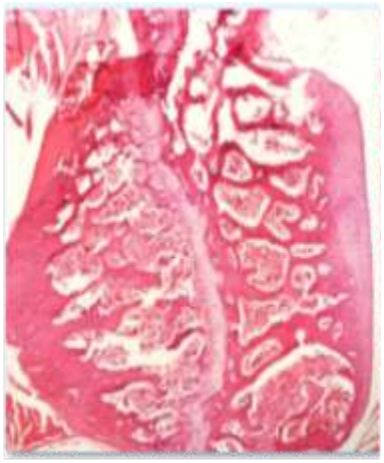

Plate 2: Photomicrograph (X400) of hind limb of arthritic untreated rat.

The bony tissue showed marked soft tissue swelling (oedematous inflammation), synovial hyperplasia with increased vascularity and inflammatory cells (lymphocytes).

Plate 3: Photomicrograph (X400) of hind limb of arthritic rat treated with diclofenac (10 mg/kg b.wt.)

The bony tissue showed synovial membrane re-establishing itself with less oedema and inflammatory cells. (lymphocytes)

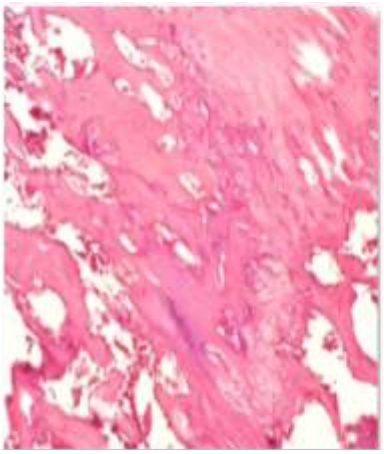

Plate 4: Photomicrograph (X400) of Hind limb of arthritic rat treated with 25 mg/kg b.wt. AEPPL.

The bony tissue showed high oedema and inflammatory cells.

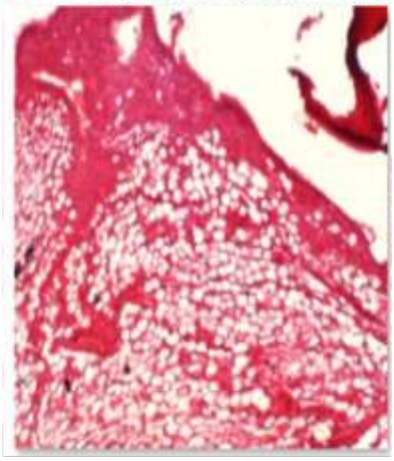

Plate 5: Photomicrograph (X400) of Hind limb of arthritic rat treated with 50 mg/kg b.wt. AEPPL.
The bony tissue showed marked reduction in oedema and inflammatory cells.

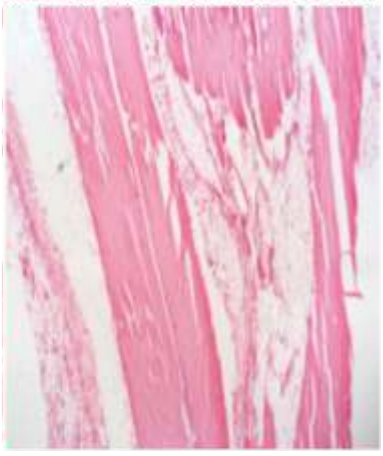

Plate 6: Photomicrograph (X400) of Hind limb of arthritic rat treated with 100 mg/kg b.wt. AEPPL.

The bony tissue showed marked reduction in oedema and inflammatory cells.

Discussion

Arthritis is characterized by synovial proliferation and inflammation in the wrist and fingers, resulting in severe disability and a reduced quality of life, especially among the elderly (Ambach *et al.,* 2022). Injection of formaldehyde into the rat paw is a biphasic process resulting to oedema (paw thickness) an early neurogenic component (localized inflammation and pain) followed by a later tissue-mediated response (Fatima, 2021). A good anti-arthritic agent should be able to suppress one or both phase(s). Tekieh *et al.* (2011) claim that the development of hind paw oedema in adjuvant-induced infection in rats is paralleled and caused by an increase in the activity of lysosomal enzymes in that region, which are involved in the breakdown of structural macromolecules in cartilage proteoglycans and connective tissue. Joint protection and suppression against synovitis are the ultimate goals for the treatment of arthritis. (Gao *et al.,* 2022). Diclofenac, an NSAID exerts its anti-inflammatory effect mainly through inhibition of COX and prostaglandin production (van Rensburg and Reuter, 2019). The 50 mg/kg b.wt. AEPPL extract used in this study achieved these goals with effects like that of the reference drug – diclofenac. Results from this study showed a significant decrease in paw thickness on the 8th and 10th day of treatment with the 50 mg/kg b.wt. AEPPL dose, which is not significantly different from the control. Though the actual mechanism of AEPPL suppressing inflammation is not known, it can be correlated with the presence of flavonoids, terpenoids, alkaloids, tannins, and saponins in suppressing inflammation and antioxidant activity (Mitropoulou *et al.,* 2023). Similarly, the presence of saponins and alkaloids has been reported to inhibit articular swelling and down-regulate the content of IL-IB and TNF-α in the inflammatory tissues of arthritic rats (Santiago *et al.,* 2021) while flavonoids are often used for their antioxidant effect against free radicals (Kumar and Pandey, 2013).

Moreover, changes in body weight have been used to gauge the progression of the condition (incidence and severity) as well as how well patients responded to anti-inflammatory drug therapy. (Makielski *et al.,* 2018). Rheumatoid arthritis is associated with weight loss and loss of lean body mass, known as rheumatoid cachexia (Hulander *et al.,* 2022). Rheumatoid cachexia is thought to be the result of cytokine-driven hypermetabolism and is a key comorbidity in rheumatoid arthritis (Efthymiou *et al.,* 2022). The loss of lean body mass is associated with decreased physical activity, and muscle strength due to alterations in metabolic activities and endurance in performing activities of daily living (Shun et al., 2021). Significant weight loss was observed upon physical assessment of arthritic animals treated with doses of AEPPL which contrasts with the treatment with standard drugs which maintain the weight of the animal throughout the treatment period. This result showed that AEPPL does not prevent weight loss and/or loss of lean body mass in contrast to the standard drug.

White blood cells (WBCs) are essential for combating illness in the body and are associated with inflammation and infectious disorders (Schmitz *et al.,* 2022). In arthritis, WBCs increase due to elevated levels of IL-1β, leading to Felty's syndrome (Yao *et al.,* 2022). However, administering higher doses of AEPPL leads to increased production of WBCs, which helps alleviate inflammation caused by formaldehyde. Red blood cells (RBCs) are the most frequently produced component of blood and are responsible for oxygen transport and CO2 waste elimination (Oladejo and Osukoya, 2021). Treatment with AEPPL, in a dose-dependent manner, enhances hematological profiles, indicating its anti-anemic and anti-arthritic effects. However, higher doses of AEPPL significantly increase platelet levels, which are essential for blood clotting. This might be a response to arthritis-linked inflammation. Due to heightened platelet signaling, platelets acquire antigen-presenting abilities, resulting in dysregulated intercellular aggregation and the formation of pathological clots (Olumuyiwa-Akeredolu *et al.,* 2019). In rheumatoid arthritis, activated platelets play a role in systemic inflammatory processes (Shlenkina *et al.,* 2019). This study observed that administering 100 mg/kg b.wt. of AEPPL resulted in a significant increase in lymphocytes, which could lead to lymphoproliferative diseases (LPDs). These diseases often affect individuals with impaired immune systems and are a notable side effect of rheumatoid arthritis (Shen and Wang, 2022). Therefore, the highest dose of AEPPL confirms the extract's effectiveness in treating rheumatoid arthritis.

One of the fundamental processes contributing to the pathophysiology of rheumatoid arthritis is oxidative stress (Zhang *et al.,* 2020). Inflamed joints produce an excessive amount of reactive oxygen species (ROS), which harms the joint tissues (Zhou *et al.,* 2023). Antioxidants can scavenge ROS and stop or lessen the harm oxidative stress causes (Wang *et al.,* 2020). Cells are equipped with antioxidant enzymes to cope with ubiquitous reactive species (superoxide anion radical and hydrogen peroxide) that are generated (Zhang and Tian, 2020). They act in a concerted manner to detoxify these noxious species (Ajiboye *et al.,* 2010). Consequently, different activities of antioxidant enzymes, such as superoxide dismutase (SOD), catalase, and reduced glutathione (GSH) have been reported to be effective in treating RA (Hamam *et al.,* 2022). During the arthritic condition, secretion of inflammatory mediators is activated, which initiates the production of ROS/free radicals in the inflamed area and causes alteration of the content of endogenous antioxidants (Zheng *et al.,* 2017).

The short-lived signaling molecule nitric oxide (NO) is crucial for several physiologic processes including inflammation. In normal circumstances, nitric oxide (NO) has been found to control T cell activities; nevertheless, excessive NO production may be a factor in T lymphocyte malfunction (Arora *et al.,* 2020). Many rheumatic disorders, including rheumatoid arthritis, have been linked to NO-dependent tissue injury (RA) (Golbahari and Froushani, 2019). There has been a notable surge in the level of NO, indicating a robust association between NO levels, disease activity, inflammatory markers, and arthritis. The administration of AEPPL has been shown to effectively diminish the quantity of NO, with the extent of reduction being dose-dependent. This implies that higher doses of AEPPL comprise compounds that act as inhibitors of NO, thereby leading to a decline in the production of this compound.

Superoxide dismutase (SOD) is responsible for the detoxification of the superoxide anion radical and its

conversion into hydrogen peroxide. Subsequently, catalase converts hydrogen peroxide into water and oxygen (Rezayian *et al.,* 2019). This study suggests that the reduced activity of SOD in untreated rats with arthritis may have led to hepatic injury due to oxidative stress caused by free radicals associated with rheumatoid arthritis. However, rats treated with AEPP at doses of 50 mg/kg b.wt and 100 mg/kg b.wt exhibited a significant increase in SOD activity compared to the untreated group. This finding is in tandem with Karatas *et al.* (2003) who attributed increased oxidative stress and low antioxidant status to RA. Catalase regulates the expression of genes involved in inflammation and restricts the production of reactive oxygen species (ROS) (El-Sohemy *et al.,* 2006). The results from this study indicate that catalase activity was significantly higher in the treatment groups, while the arthritic untreated group exhibited low activity. The administration of 50 mg/kg b.wt of AEPPL restored catalase activity to normal levels, with a more pronounced effect observed with 100 mg/kg b.wt of AEPPL. This suggests that the constituents of Paullinia pinnata may have an antioxidant role by reducing ROS production in rheumatoid arthritis.

In biological systems, reduced glutathione (GSH) acts as the primary defense against free radicals and plays a crucial role in preventing tissue and organ damage (Ujowundu *et al.,* 2012). GSH directly inhibits lipid oxidation by forming an unstable sulfhydryl radical with free radicals (Ali *et al.,* 2020). The decrease in liver GSH concentration in arthritic untreated rats was reversed in a dose-dependent manner with AEPPL treatment. This indicates a possible effect on the de novo synthesis and/or regeneration of GSH.

Lipid peroxidation (LPO) is a common indicator of lipid damage (Gianazza *et al.,* 2020), and malondialdehyde (MDA) (an indicator of LPO) is elevated during arthritis and is a significant marker of inflammatory disease. Also, Nitric Oxide (NO) reacts with superoxide anion to form peroxynitrite which contributes to oxidative stress by way of lipid peroxidation (Negre-Salvayre *et al.,* 2022). Increased MDA indicates increased lipid peroxidation which could have resulted from depletion of antioxidant enzymes. The maintenance of the MDA concentration of AEPP-treated rats close to the concentration of control rats implies that AEPP restored or increased the level of antioxidants. This shows that AEPPL can salvage dyslipidemia in arthritic rats.

Additionally, the study found that AEPPL treatment effectively reduced inflammation in the synovial lining of the hind limb joint of the 100 mg AEPPL-treated rats. This could lead to the regeneration of cartilage and a decrease in inflammatory cells, indicating that it effectively addresses the underlying causes of the disease.

Conclusion

The findings of this study indicate that the administration of an oral aqueous extract of P. pinnata to Wistar rats demonstrates potent antioxidant and anti-arthritic properties. Notably, the extract dosage of 100 mg/kg body weight proved to be particularly efficacious. These results provide further validation for the traditional utilization of P. pinnata leaf as a therapeutic agent for its antioxidant and anti-arthritic effects. The observed outcomes can potentially be attributed to the presence of phytoconstituents previously documented in the literature.

Conflict of interest: The authors declare that there is no conflict of interest.

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