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Exploring the anti-arthritic and anti- inflammatory activity of ethanol extract of *Mimosa pudica* leaves against complete Freund's adjuvant (CFA) induced arthritis in Wistar albino rats

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Abstract

The study explores the potential anti-arthritic and anti-inflammatory effects of an ethanol extract from *Mimosa pudica* leaves in a rat model of arthritis induced by Complete Freud's Adjuvant (CFA). The extract's efficacy was evaluated by measuring key markers associated with inflammation and arthritis. The results revealed a significant reduction in inflammation and arthritis-related symptoms in the treatment group, suggesting the extract's strong anti-inflammatory properties. The findings highlight the therapeutic potential of *Mimosa pudica*s a natural remedy for arthritis, which could be attributed to its active phytochemicals like flavonoids and alkaloids. Further studies are needed to clarify the underlying mechanisms and explore their possible use in treating human arthritis.

Keyword: *Mimosa pudica*; Anti-arthritic activity; Anti-inflammatory effects; Complete Freund's Adjuvant (CFA); Wistar albino rats; Ethanol extract; Phytochemicals; Flavonoids and alkaloids; Rheumatoid arthritis model

1. Introduction

Inflammation is a critical immune response that protects tissues from damage caused by pathogens, injuries, or irritants. This process activates immune cells, releases signaling molecules, and increases blood flow to affected areas, helping the body to heal. While acute inflammation is beneficial, prolonged or chronic inflammation can be harmful and is linked to conditions such as arthritis, cardiovascular diseases, and certain cancers. Managing inflammation is crucial for preventing disease progression, with treatments often including anti-inflammatory drugs like NSAIDs and corticosteroids, which target specific pathways to reduce symptoms. Newer therapies, such as biologics and Disease-Modifying Anti-Rheumatic Drugs (DMARDs), address inflammation at the immune level, offering more targeted solutions. Additionally, natural agents, including omega-3 fatty acids and turmeric, have gained attention for their anti-inflammatory properties, with potential health benefits and lower side effects. Exploring synthetic and natural anti-inflammatory agents is essential for advancing treatments for inflammation-driven diseases.

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1.1. Types Of Inflammation

Table 1 Disease course of the various types of Inflammation:[6]

Type	Disease Course
Acute Inflammation	Acute inflammation is a rapid and short-term response that typically lasts from a few hours to a few days. It occurs as a defense mechanism in response to injury or infection and is characterized by redness, swelling, heat, pain, and sometimes a temporary loss of function. Acute inflammation is crucial for healing, allowing immune cells to reach the affected area and begin repair quickly.
Chronic Inflammation	Chronic inflammation is a prolonged, persistent response that can last weeks, months, or even years. It often arises when acute inflammation fails to resolve the underlying issue or due to continuous exposure to an irritant. Unlike acute inflammation, chronic inflammation may not display obvious symptoms but can lead to tissue damage over time, contributing to conditions such as rheumatoid arthritis, cardiovascular diseases, and some cancers.
Subacute Inflammation	This intermediate form lasts longer than acute inflammation but is not as prolonged as chronic inflammation. It usually lasts from several days to a few weeks. It is a transitional phase where the inflammatory response is still active but starting to resolve. Subacute inflammation can occur in situations where an infection is still present but not as severe, or in the healing phase of an acute injury.

1.2. Pathogenesis

The pathogenesis of inflammation begins with the immune system's recognition of harmful stimuli, such as pathogens or damaged cells, by immune receptors that detect molecular patterns associated with infection or injury. Upon activation, immune cells release pro-inflammatory mediators—like cytokines, chemokines, histamines, and prostaglandins—that increase blood vessel dilation and permeability, allowing immune cells, fluids, and proteins to enter affected tissues. Leukocytes, primarily neutrophils and monocytes, migrate to the site, where they eliminate pathogens and debris through phagocytosis while amplifying the inflammatory response. The process concludes with resolution, where anti-inflammatory mediators, such as lipoxins, actively promote tissue repair and restore balance. If unresolved, however, inflammation may become chronic, contributing to long-term tissue damage and diseases like rheumatoid arthritis and atherosclerosis.

2. Materials and methods [1]

2.1. Animals

The animal experimental protocol was approved by the Institutional Animal Ethical Committee of Aadhibhagavan College of Pharmacy. Healthy Wister Albino rats 6-8 weeks old, weighing 200- 250 gm were used for the study. Animals were housed in polypropylene cages (5 animals per cage) maintained under standard conditions (12-hour light, 12-hour dark cycle; 27 ± 3 ℃, 35-50 % humidity), fed with standard pellet diet and free access to water (ad libitium), and allowed to acclimatize for 7 days before starting the experiment.

2.2. Animals and experimental design [2][3]

The animals were randomly divided into five groups, each containing six animals (n=6). Group I served as the normal control and received no CFA injection, while Group II acted as the CFA control, receiving only the vehicle without any treatment. Group III received a reference standard dose of Dexamethasone at (5 mg/kg/day). Groups IV and V were treated with *M. pudica* extract at low doses of 100 mg/kg/day and high doses of 500 mg/kg/day, respectively. Treatments for Groups III to V, as well as the CFA control group, were administered orally each morning between 8 and 9 am, with measurements recorded at 2 pm.

2.3. Induction of arthritis [5]

Experimental immunological arthritis was induced following a modified protocol based on the method described by reference 19, with adjustments in group classification. Briefly, the hind limbs of all animals, except those in Group I, were shaved and sterilized using 70% (v/v) alcohol. A 0.1 mL injection of CFA containing 10 mg/mL heat-killed *Mycobacterium tuberculosis* was administered into the sub-plantar region of the left hind paw of each animal under

mild diethyl ether anesthesia. The day of the CFA injection was designated as day 0. Daily oral doses of the vehicle, M.pudica extract, or Dexamethasone were initiated on day 0 and continued until day 21 post-injection.

2.4. Assessment of arthritis

2.4.1. Effect Of AEMP On CFA Induced Paw Edema Volume:[12][13]

The edema in both hind paws was measured on days 0, 4, 8, 12, 16, 20, 24, and 28 after CFA injection using a digital micrometer gauge. The percentage increase in paw edema was calculated using the following formula:

$$\text{Percentage Increase in Paw Edema} = \text{Volume on Day X} - \text{Paw Volume on Day 0} / \text{Paw Volume on Day 0Paw} \times 100$$

The mean of paw thickness at a particular time. The results were statistically compared to the CFA-control group

2.4.2. Effect of AEMP on arthritic score:[7][8]

Arthritis in Wistar albino rats can be induced through various methods, such as injecting adjuvants like Complete Freund's Adjuvant (CFA) or Monoiodoacetate (MIA) into the joints or using autoimmune models like collagen-induced arthritis (CIA) and adjuvant-induced arthritis (AIA). The arthritic condition is then assessed by evaluating joint swelling, erythema (redness), and deformity. Measurements for joint swelling are taken with a caliper or ruler, while erythema is visually assessed based on redness around the joint, and deformity is evaluated by observing changes in joint structure or mobility. A scoring system is used to quantify these parameters, typically assigning scores from 0 to 3 for each, where 0 represents no symptom presence, and 3 indicates severe presentation.

2.4.3. Effect of AEMP on alkaline phosphate:[9][10]

Phosphatases are enzymes that catalyze the release of phosphoric acid from specific monophosphoric esters, resulting in the production of phenol and disodium phosphate. Under alkaline conditions, the liberated phenol interacts with phosphomolybdate in the Folin-Ciocalteu reagent, forming a blue complex measurable at 620 nm. The procedure involves preparing a carbonate-bicarbonate buffer (pH 10) and creating a phenol stock solution by dissolving phenol in HCl and water, and then diluting it to obtain a working standard. Sodium phenyl phosphate is dissolved in bicarbonate buffer as the substrate. In the assay, phenol standards, along with water for the blank, are mixed in tubes with Folin-Ciocalteu reagent, and enzyme activity is assessed by adding the buffered substrate and incubating for 5 minutes. After adding serum to specific tubes and centrifuging, the supernatant is collected, mixed with distilled water and 15% Na_2CO_3, and the blue color intensity is read at 640 nm, with enzyme activity expressed in IU/L.

2.4.4. Effect of AEMP on serum glutamicoxaloacetic transaminase (SGOT):[8][9]

SGOT facilitates the transfer of an amino group from L-aspartate to α-ketoglutarate, producing oxaloacetate and glutamate. The resulting oxaloacetate is reduced to malate by malate dehydrogenase and NADH, which appears brown in an alkaline medium. The rate of NADH reduction is measured with a commercial kit based on the method of Reitman and Frankel (1957). For the assay, a 0.1 M phosphate buffer (pH 7.4) is prepared by mixing solutions of disodium hydrogen phosphate and potassium dihydrogen phosphate. A buffered substrate solution of aspartic acid and α-ketoglutarate in phosphate buffer is used, along with DNPH (2,4-dinitrophenylhydrazine) in HCl and NaOH for color development. The assay procedure involves incubating the buffered substrate, adding serum, and then incubating with DNPH and NaOH before measuring the optical density using a green filter. Results are expressed in units per liter of serum.

2.4.5. Effect of AEMP on serum glutamic pyruvic transaminase (SGPT):[10][11][12]

SGPT (Alanine Transaminase) catalyzes the transfer of an amino group from L-alanine to α-ketoglutarate, producing glutamate and pyruvate. The pyruvate generated is subsequently reduced to lactate by lactate dehydrogenase and NADH, with the rate of NADH decrease measured in the reaction. For this assay, a 0.1 M phosphate buffer (pH 7.4) is prepared by combining disodium hydrogen phosphate and potassium dihydrogen phosphate solutions. A buffered substrate of alanine and α-ketoglutarate in phosphate buffer is used along with DNPH (2,4-dinitrophenylhydrazine) in HCl, NaOH, and a pyruvate standard. The assay procedure involves incubating the buffered substrate, adding serum, and incubating with DNPH and NaOH before measuring the optical density (OD) using a green filter. Results are expressed in IU/L of serum.

2.4.6. Effect of AEMP on urea:[22][23]

The urea determination process involves measuring the concentration of urea, a nitrogenous compound typically present in biological samples like blood or urine. This method relies on the enzymatic hydrolysis of urea by urease, which produces ammonia and carbon dioxide. The ammonia formed is directly proportional to the urea concentration in the sample and can be quantified either spectrophotometrically or visually with an indicator reagent. This assay is frequently used in clinical laboratories to assess kidney function and monitor nitrogen metabolism. Key reagents include urease to catalyze the reaction, an alkaline buffer to maintain optimal pH, and an indicator reagent (such as phenol red) to signal ammonia presence. The procedure involves preparing urea standards, adding urease and buffer, incubating the reaction, and then measuring the color change or absorbance. By comparing sample results to a standard curve, the urea concentration in each sample can be accurately determined.

2.4.7. Effect of AEMP on lipid peroxidation *(LPO):[24][25][26]*

Lipid peroxidation is an oxidative process that breaks down lipids, especially unsaturated fatty acids, through reactive oxygen species (ROS) or free radicals, producing lipid hydroperoxides and other reactive compounds that can harm cells and impair function. To evaluate lipid peroxidation in biological samples, such as tissues or fluids, samples are homogenized or lipids are extracted with specific solvents, taking care to minimize oxidation. Quantification of lipid peroxidation products can be achieved using several methods: the TBARS assay, which measures malondialdehyde (MDA) as an indicator of lipid damage; fluorescent probes like BODIPY, which detect lipid peroxides; or gas chromatography-mass spectrometry (GC-MS) for precise analysis of peroxidation products. Reagents include thiobarbituric acid (TBA) for the TBARS assay, hydrochloric acid and trichloroacetic acid (TCA) for sample acidification and protein precipitation, antioxidants to prevent further oxidation, and organic solvents for extraction. Results are then compared across samples to evaluate oxidative stress or lipid peroxidation levels under different conditions.

2.4.8. Effect of AEMP on creatinine:[15][16]

Creatinine is a byproduct of creatine metabolism in muscles, produced at a steady rate and excreted by the kidneys into urine. Blood and urine creatinine levels are commonly measured as indicators of kidney function, with elevated blood creatinine suggesting potential kidney impairment. In the procedure, a blood or urine sample is collected, handled, and stored to prevent creatinine degradation. Blood samples may need centrifugation to isolate serum or plasma, while urine samples might require dilution for optimal detection. Quantification of creatinine can be achieved through enzymatic or colorimetric assays. The enzymatic assay uses creatininase and creatinase enzymes to produce creatine and ammonia, which are then detected via enzyme-coupled reactions. The colorimetric assay, using alkaline picrate, forms a yellow-orange complex with creatinine, measurable spectrophotometrically. Results are calculated based on absorbance or reaction rates and interpreted against reference ranges to assess kidney function. Key reagents include creatinine standard solution for calibration, enzymes for enzymatic assays, alkaline picrate for colorimetric assays, and buffer solutions for pH stability, ensuring precise measurement for clinical diagnostics.

2.4.9. Effect of AEMP on catalyse*:[17][18]*

In this assay, dichromate in acetic acid is reduced to chromic acetate when heated in the presence of hydrogen peroxide, with perchromic acid forming an unstable intermediate. The resulting chromic acetate is measured at 590 nm. To assess catalase activity, hydrogen peroxide (H_2O_2) is allowed to be degraded by catalase over various time intervals. The reaction is halted at specific time points by adding a dichromate-acetic acid mixture, allowing for the remaining H_2O_2 to be quantified colorimetrically. For this procedure, reagents include 0.01 M phosphate buffer (pH 7), 2 M H_2O_2, 5% potassium dichromate, and a dichromate-acetic acid solution prepared by mixing potassium dichromate with glacial acetic acid in a 1:3 ratio. In the assay, 1 mL of phosphate buffer, 0.1 mL homogenate, and 0.4 mL H_2O_2 are combined. After 30 seconds, 2 mL of the dichromate-acetic acid reagent is added to stop the reaction. Samples are then heated in a boiling water bath for 10 minutes, cooled, and read at 620 nm. A control without enzyme is used, and results are expressed in terms of moles of H_2O_2 decomposed per minute per mg of tissue protein or per mL of serum.

2.4.10. Effect of AEMP on superoxide dismutase (sod):[19][20]

This assay measures the inhibition of NADH-phenazine methosulfate-nitroblue tetrazolium (NBT) formazan formation. Initiated by adding NADH, the reaction proceeds for 90 seconds before being halted by glacial acetic acid. The resulting color is then extracted into an *n*-butanol layer and quantified spectrophotometrically at 520 nm. Key reagents include sodium pyrophosphate buffer (pH 8.3), PMS, NBT, NADH, glacial acetic acid, *n*-butanol, chloroform, and ethanol. For the procedure, 0.5 mL of liver homogenate is diluted, mixed with chilled ethanol and chloroform, and centrifuged. The supernatant is used in an assay mixture containing the buffer, PMS, NBT, and NADH, brought to a final volume of 3 mL. The reaction mixture, incubated at 30°C for 90 seconds, is then treated with glacial acetic acid and mixed with *n*-

butanol. The chromogen intensity in the butanol layer is measured at 560 nm, with a butanol blank as a reference. One enzyme unit is defined as the activity causing 50% inhibition of NBT reduction per minute, reported as unit/mg protein for tissue or unit/mL for serum.

2.4.11. Effect of AEMP on reduced glutathione (gsh):[26][27]

Glutathione Reductase (GR) is an enzyme that facilitates the conversion of oxidized glutathione (GSSG) to its reduced form (GSH) using NADPH as a cofactor. In this assay, a reaction mixture is prepared containing phosphate buffer (pH 7.0–8.0), NADPH, GSSG, and the sample with GR. The mixture is incubated at a suitable temperature to enable the reaction, after which the decrease in absorbance at 340 nm is measured spectrophotometrically. This decrease correlates with the activity of GR, as it reflects the consumption of NADPH during the reduction of GSSG. By plotting the absorbance changes against a standard curve created with known NADPH concentrations, the enzymatic activity of GR in the sample can be quantified.

2.5. Data analysis

All the data was expressed as mean ± SEM and statistically analyzed by GraphPad Prism software using one-way ANOVA followed by post hoc Dunnett-t test at different variance levels.

3. Results

Table 2 Effect of AEMP On Complete Freund's Adjuvant (CFA) Induced Paw Edema Volume

S.No	Groups	Time (in Hours)					
		0 hr	**1 hr**	**2 hr**	**3hr**	**4hr**	**5 hr**
1	Control	0.834 ± 0.053	1.01 ± 0.039	1.022 ± 0.041	0.902 ± 0.042	0.964 ± 0.026	1.022 ± 0.0412
2	Negative	1.010 ±0.039 a^{****}	1.986 ± 0.042 a^{****}	2.124 ± 0.084 a^{****}	2.090 ±0.059 a^{****}	2.200 ±0.142 a^{****}	1.860 ±0.222 a^{****}
3	Standard	1.022 ±0.041 $a^{****}b^{****}$	2.240 ± 0.116 $a^{****}b^{ns}$	1.910 ± 0.057 $a^{****}b^{ns}$	1.550 ± .059 $a^{****}b^{****}$	1.212 ±0.052 $a^{ns}b^{****}$	0.926 ±0.055 $a^{ns}b^{****}$
4	Low Dose	0.902 ±0.042 $a^{****}b^{****}c^{****}$	2.470 ±0.072 $a^{****}b^{****}c^{ns}$	2.112 ±0.059 $a^{****}b^{ns}c^{ns}$	1.844 ±0.053 $a^{****}b^{ns}c^{*}$	1.650 ±0.050 $a^{****}b^{****}c^{***}$	1.380 ±0.037 $a^{**}b^{****}c^{***}$
5	High Dose	0.964 ±0.026 $a^{****}b^{****}c^{****}$	2.560 ± $a^{****}b^{****}c^{****}$	1.946 ± 0.042 $a^{****}b^{ns}c^{ns}$	1.492 ±0.033 $a^{****}b^{****}c^{ns}$	1.044 ±0.025 and $b^{****}c^{ns}$	0.898 ±0.031 $a^{ns}b^{****}c^{ns}$

The data, expressed as Mean ± SEM with *p<0.05, **p<0.01, ***p<0.001, ***p<0.0001, and ****p<0.00001, were analyzed using one-way ANOVA followed by Dunnett's test across six groups (n=6). Statistical comparisons include: Group II versus Groups III, IV, and V Statistical significance is denoted as follows: "ns" for non-significant differences, with a gradient of significance indicated by increasing asterisks for progressively lower p-values, where fewer asterisks represent less significant differences.

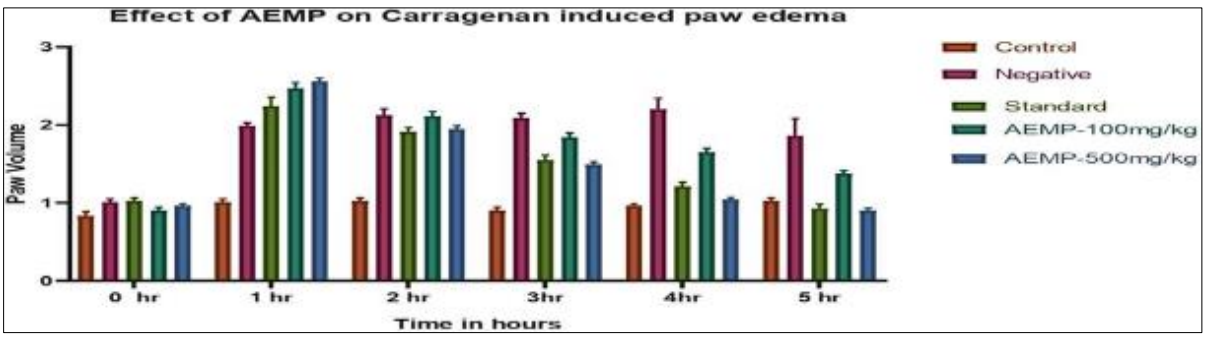

Figure 1 Effect of AEMP On Complete Freund's Adjuvant (CFA) Induced Paw Edema Volume

Table 3 Effect of AEMP on arithiritc score, ALP, SGOT, SGPT, UREA

S.No	GROUPS	Arithiritic score	ALP	SGOT	SGPT	UREA
1	Control	918.7 ± 1.031	119.9 ± 1.088	130.5 ± 7.054	155.5 ± 3.712	333.5 ± 0.8839
2	Negative Control	3.800 ± 0.23	330.5 ± 7.966	428.9 ± 7.418	364.2 ± 2.934	380.5 ± 7.054
3	Standard Control	1.200 ± 0.24 ****	133.4 ± 2.082****	126.7 ± 1.548 ****	146.5 ± 1.422 ****	298.5 ± 1.973****
4	AEMP (100 mg/kg)	2.400 ± 0.24 ***	235.6 ± 3.517 ****	325.5 ± 7.054 ****	191.5 ± 2.885 ****	345.4 ± 3.508 ****
5	AEMP (500 mg/kg)	1.400 ± 0.21 ****	146.7 ± 1.548 ***	166.7 ± 1.548 ****	166.6 ± 2.821 ****	315.5 ± 3.548****

The data, expressed as Mean ± SEM with *p<0.05, **p<0.01, ***p<0.001, ***p<0.0001, and ****p<0.00001, were analyzed using one-way ANOVA followed by Dunnett's test across six groups (n=6). Statistical comparisons include: Group II versus Groups III, IV, and V Statistical significance is denoted as follows: "ns" for non-significant differences, with a gradient of significance indicated by increasing asterisks for progressively lower p-values, where fewer asterisks represent less significant differences. (ALP- Alkaline Phosphate, SGOT- Serum Glutamic Oxalo Acetic Transaminase, SGPT- Serum Glutamic Pyruvic Transaminase).

Table 4 Effect of AEMP on LPO, Creatinine, CAT, SOD, GSH

Type	Disease Course
Acute Inflammation	Acute inflammation is a rapid and short-term response that typically lasts from a few hours to a few days. It occurs as a defense mechanism in response to injury or infection and is characterized by redness, swelling, heat, pain, and sometimes a temporary loss of function. Acute inflammation is crucial for healing, allowing immune cells to reach the affected area and begin repair quickly.
Chronic Inflammation	Chronic inflammation is a prolonged, persistent response that can last weeks, months, or even years. It often arises when acute inflammation fails to resolve the underlying issue or due to continuous exposure to an irritant. Unlike acute inflammation, chronic inflammation may not display obvious symptoms but can lead to tissue damage over time, contributing to conditions such as rheumatoid arthritis, cardiovascular diseases, and some cancers.
Subacute Inflammation	This intermediate form lasts longer than acute inflammation but is not as prolonged as chronic inflammation. It usually lasts from several days to a few weeks. It is a transitional phase where the inflammatory response is still active but starting to resolve. Subacute inflammation can occur in situations where an infection is still present but not as severe, or in the healing phase of an acute injury.

The data, expressed as Mean ± SEM with *p<0.05, **p<0.01, ***p<0.001, ***p<0.0001, and ****p<0.00001, were analyzed using one-way ANOVA followed by Dunnett's test across six groups (n=6). Statistical comparisons include: Group II versus Groups III, IV, and V Statistical significance is denoted as follows: "ns" for non-significant differences, with a gradient of significance indicated by increasing asterisks for progressively lower p-values, where fewer asterisks represent less significant differences. (LPO- Lipid Peroxidation, CAT- Catalyse, SOD- Superoxide Dimutase, GSH- Reduced Glutathione).

3.1. Effect of AEMP on complete freund's adjuvant-induced paw edema volume (5HR)

In this study, we evaluated the effects of various treatment groups compared to a Control group. The Negative group exhibited a significant increase in Time compared to the Control ($p < 0.00001$), indicating a delay in the desired effect. Conversely, both the Standard ($p < 0.00001$) and High Dose ($p < 0.00001$) groups displayed significantly reduced Time, with the Standard group closely mirroring the Control, suggesting a potential therapeutic benefit. The Low Dose group also demonstrated a notable change in Time relative to the Control ($p < 0.00001$). Additionally, when comparing the Negative group to the Standard, low-dose, and High-dose groups, all showed significantly decreased Time ($p < 0.00001$), highlighting the efficacy of these treatments. However, in comparing the Standard group with the Low and high-dose groups, the Low Dose group showed a significant increase in Time ($p < 0.001$), while the High Dose group also exhibited a similar increase ($p < 0.0001$), indicating that higher doses may not significantly enhance therapeutic effects relative to lower doses.

3.2. Effect of AEMP on arthritic score

In our investigation, we compared the arthritic scores across various treatment groups to assess the efficacy of AEMP. The Negative Control group (Group II) exhibited significantly higher arthritic scores than the Control group (Group I) ($p < 0.00001$), highlighting a notable adverse effect. Both the Standard Control (Group III) ($p < 0.001$) and the AEMP treatments at 100 mg/kg (Group IV) ($p < 0.00001$) and 500 mg/kg (Group V) ($p < 0.00001$) demonstrated significant reductions in arthritic scores compared to the Control group, indicating their potential therapeutic effects. Furthermore, when comparing the Negative Control group to the Standard Control and both doses of AEMP, all groups exhibited significantly lower arthritic scores ($p < 0.00001$), reinforcing the effectiveness of AEMP in mitigating arthritis severity. Notably, while AEMP at 100 mg/kg (Group IV) showed a significant decrease in arthritic score compared to the Standard Control ($p < 0.00001$), the higher dose of AEMP at 500 mg/kg (Group V) did not yield a significant difference ($p > 0.05$) when compared to the Standard Control, suggesting that the increased dosage may not provide additional therapeutic benefits over the standard treatment.

3.3. Effect of AEMP on alkaline phosphate

In our analysis of ALP levels across different treatment groups, we observed significant differences when comparing Group I (Control) with Groups II, III, IV, and V. The Negative Control group (Group II) exhibited a significant increase in ALP levels compared to the Control ($p < 0.00001$), indicating an adverse effect. In contrast, Groups III ($p < 0.00001$), IV ($p < 0.00001$), and V ($p < 0.00001$) all showed significantly reduced ALP levels compared to the Control group, with Group V notably approaching Control levels, suggesting a potential dose-dependent effect of AEMP. When examining the Negative Control against Groups III, IV, and V, all displayed significantly lower ALP levels ($p < 0.00001$), reinforcing the potential therapeutic effect of AEMP. Furthermore, when comparing Group III (Standard Control) with Groups IV and V, Group IV showed a lesser decrease in ALP levels ($p < 0.00001$). At the same time, Group V exhibited a more significant reduction ($p < 0.01$). This indicates that while higher doses of AEMP may not substantially enhance therapeutic effects compared to lower doses, they still contribute to a notable decrease in ALP levels.

3.4. Effect of AEMP on serum glutamic oxaloacetic transaminase (SGOT)

In this study, we assessed SGOT levels across different treatment groups, where Group I (Control) was compared to Groups II, III, IV, and V. The Negative Control group (Group II) exhibited a significant increase in SGOT levels compared to the Control ($p < 0.00001$), suggesting potential liver damage. In contrast, Groups III ($p < 0.00001$), IV ($p < 0.00001$), and V ($p < 0.00001$) showed significantly lower SGOT levels compared to the Control group, with Group V demonstrating levels that were notably closer to those of the Control. This trend indicates a potential dose-dependent effect of AEMP in reducing SGOT levels. Additionally, when comparing the Negative Control to Groups III, IV, and V, all groups exhibited significantly decreased SGOT levels ($p < 0.00001$), reinforcing the therapeutic potential of AEMP in mitigating liver damage associated with the Negative Control condition.

3.5. Effect of AEMP on serum glutamic pyruvic transaminase (SGPT)

In our investigation of SGPT levels among various treatment groups, we compared Group I (Control) with Groups II, III, IV, and V. The Negative Control group (Group II) exhibited a significant increase in SGPT levels relative to the Control ($p < 0.00001$), indicating potential liver damage. Conversely, Groups III ($p < 0.00001$), IV ($p < 0.00001$), and V ($p < 0.00001$) demonstrated significantly different SGPT levels compared to the Control, with Group V notably approaching Control levels, suggesting a potential dose-dependent effect of AEMP in reducing SGPT levels. Furthermore, when comparing the Negative Control group with Groups III, IV, and V, all exhibited significantly decreased SGPT levels ($p < 0.00001$), underscoring the therapeutic potential of AEMP in alleviating liver damage. Additionally, in comparing Group III (Standard Control) with Groups IV and V, Group IV showed a lesser reduction in SGPT levels ($p < 0.00001$), while Group V demonstrated a more substantial decrease ($p < 0.001$). This indicates that while the higher dose of AEMP may not provide a significantly greater therapeutic benefit compared to the lower dose, it still effectively reduces SGPT levels associated with liver injury.

3.6. Effect of AEMP on urea

In this study, we analyzed urea levels among various treatment groups, where Group I (Control) was compared with Groups II, III, IV, and V. The Negative Control group (Group II) displayed a significant increase in urea levels compared to the Control ($p < 0.00001$), indicating potential renal dysfunction. In contrast, Groups III ($p < 0.001$) and V ($p < 0.01$) exhibited significantly different urea levels relative to the Control, while Group IV showed no significant difference ($p > 0.05$), indicating urea levels similar to those of the Control and suggesting a potential dose-dependent effect of AEMP. When comparing the Negative Control with Groups III, IV, and V, all groups showed significantly decreased urea levels (Group III: $p < 0.00001$; Group IV: $p < 0.05$; Group V: $p < 0.001$), highlighting the therapeutic potential of AEMP in

mitigating renal dysfunction. Additionally, when examining Group III against Groups IV and V, Group IV demonstrated a lesser increase in urea levels ($p < 0.00001$), while Group V showed a decrease ($p < 0.05$). This finding indicates that the higher dose of AEMP may not provide a significant enhancement in therapeutic effect compared to the lower dose, although both doses effectively contribute to reducing urea levels associated with renal impairment.

3.7. Effect of AEMP on lipid peroxidation (LPO)

In this study, we evaluated lipid peroxidation levels across various treatment groups, with Group I (Control) compared to Groups II, III, IV, and V. The Negative Control group (Group II) exhibited a significant increase in lipid peroxidation levels compared to the Control ($p < 0.00001$), indicating oxidative stress. In contrast, Groups III ($p < 0.001$), IV ($p < 0.00001$), and V ($p < 0.001$) all demonstrated significantly different lipid peroxidation levels relative to the Control, with Group IV showing levels similar to the Control, suggesting a potential dose-dependent effect of AEMP. When comparing the Negative Control to Groups III, IV, and V, all groups exhibited significantly decreased lipid peroxidation levels (Group III: $p < 0.01$; Group IV: $p < 0.00001$; Group V: $p < 0.01$), indicating the antioxidant potential of AEMP. Furthermore, in the comparison between Group III and Groups IV and V, Group IV showed a lesser increase in lipid peroxidation levels ($p < 0.00001$), while Group V demonstrated a similar decrease ($p < 0.05$). This finding implies that although the higher dose of AEMP may not significantly enhance the antioxidant effect compared to the lower dose, both doses effectively contribute to reducing oxidative stress indicated by lipid peroxidation levels.

3.8. Effect of aemp on creatinine

In this research, we assessed creatinine levels among different treatment groups, with Group I (Control) compared to Groups II, III, IV, and V. The Negative Control group (Group II) exhibited a significant increase in creatinine levels compared to the Control ($p < 0.00001$), indicating renal dysfunction. Conversely, Groups III ($p < 0.00001$), IV ($p < 0.0001$), and V ($p < 0.01$) all showed significantly different creatinine levels relative to the Control, with Group V particularly notable for having creatinine levels similar to the Positive Control, suggesting a potential dose-dependent effect of AEMP. Additionally, when comparing the Negative Control with Groups III, IV, and V, all groups exhibited significantly decreased creatinine levels (Group III: $p < 0.00001$; Group IV: $p < 0.00001$; Group V: $p < 0.05$), indicating the protective potential of AEMP against renal dysfunction. Furthermore, in comparing Group III with Groups IV and V, Group IV showed a lesser increase in creatinine levels ($p < 0.01$), while Group V demonstrated a similar decrease ($p < 0.001$). This finding implies that while the higher dose of AEMP may not significantly enhance the protective effect against renal dysfunction compared to the lower dose, both doses contribute effectively to mitigating creatinine elevation associated with renal impairment.

3.9. Effect of AEMP on catalyse

In this study, we investigated catalase activity across various treatment groups, with Group I (Control) compared to Groups II, III, IV, and V. The Negative Control group (Group II) exhibited a significant increase in catalase activity compared to the Control ($p < 0.00001$), suggesting a potential oxidative stress response. In contrast, Groups III ($p < 0.001$), IV ($p < 0.0001$), and V ($p < 0.05$) demonstrated significantly different catalase activity levels relative to the Control, with Group IV notably exhibiting catalase activity similar to the Positive Control, which implies a potential dose-dependent effect of AEMP. Furthermore, when comparing the Negative Control with Groups III, IV, and V, all groups showed significantly decreased catalase activity (Group III: $p < 0.01$; Group IV: $p < 0.00001$; Group V: $p < 0.001$), indicating a potential protective effect of AEMP against oxidative stress. Additionally, in comparisons between Group III and Groups IV and V, Group IV showed a lesser increase in catalase activity ($p < 0.05$), while Group V exhibited a more substantial decrease ($p < 0.00001$). This suggests that while the higher dose of AEMP may not provide a significant enhancement in the protective effect against oxidative stress compared to the lower dose, both dosages are effective in reducing catalase activity associated with oxidative damage.

3.10. Effect of AEMP on superoxide dismutase (SOD)

In this investigation, we analyzed superoxide dismutase (SOD) activity across multiple treatment groups, with Group I (Control) compared to Groups II, III, IV, and V. The Negative Control group (Group II) exhibited a significant decrease in SOD activity compared to the Control ($p < 0.00001$), indicating a potential decline in antioxidant defense mechanisms. Conversely, Groups III ($p < 0.00001$), IV ($p < 0.00001$), and V ($p < 0.00001$) displayed significantly different SOD activity levels relative to the Control, with Group V notably showing SOD activity similar to the Positive Control, suggesting a possible dose-dependent effect of AEMP. Additionally, when comparing the Negative Control with Groups III, IV, and V, all groups demonstrated significantly increased SOD activity (Group III: $p < 0.00001$; Group IV: $p < 0.00001$; Group V: $p < 0.00001$), indicating that AEMP may aid in restoring antioxidant defenses. In further comparisons between Group III and Groups IV and V, Group IV they exhibited a lesser increase in SOD activity ($p < 0.00001$), while Group V showed a similar increase ($p < 0.00001$). This suggests that while the higher dose of AEMP may not significantly enhance the

restoration of antioxidant defense compared to the lower dose, both dosages effectively contribute to improving SOD activity following oxidative stress.

3.11. Effect of AEMP on reduced glutathione (GSH)

In this study, we examined the levels of glutathione (GSH) across various treatment groups, with Group I (Control) being compared to Groups II, III, IV, and V. The Negative Control group (Group II) showed a significant decrease in GSH levels compared to the Control ($p < 0.00001$), indicating a potential depletion of antioxidant reserves. In contrast, Groups III ($p < 0.001$), IV ($p < 0.00001$), and V ($p < 0.01$) all displayed significantly different GSH levels when compared to the Control, with Group V notably showing GSH levels comparable to the Positive Control, suggesting a potential dose-dependent effect of AEMP. When comparing the Negative Control with Groups III, IV, and V, all groups exhibited significantly increased GSH levels (Group III: $p < 0.001$; Group IV: $p < 0.00001$; Group V: $p < 0.01$), indicating a potential restoration of antioxidant reserves facilitated by AEMP. Furthermore, in comparisons between Group III and Groups IV and V, Group IV exhibited a lesser increase in GSH levels ($p < 0.001$), while Group V demonstrated a similar increase ($p < 0.01$). This finding implies that the higher dose of AEMP may not significantly enhance the restoration of antioxidant reserves compared to the lower dose, although both dosages effectively contribute to replenishing GSH levels following oxidative stress.

3.12. Histopathological report

The histological evaluation of different treatment groups revealed distinct cellular and tissue changes associated with arthritis. In the Normal Control group, healthy cellular structures were observed, with normal stratification of the epidermis and intact collagen fibers and blood vessels. In contrast, the Arthritis Control group displayed significant pathological changes, including hyperplasia and thickening of the skin layers, alongside extensive inflammation in the subcutaneous tissue, indicating a robust inflammatory response. The Standard Group treated with Dexamethasone showed a marked reduction in inflammation, abnormal cell proliferation, and infiltration of inflammatory cells, suggesting its effectiveness in managing arthritis-related changes. Meanwhile, the AEMP group receiving 100 mg/kg demonstrated decreased synovial joint destruction and reduced neutrophil infiltration, indicating a protective effect. The higher dose of AEMP at 500 mg/kg further improved outcomes, with less synovial damage, reduced inflammation, and fewer joint erosions compared to the Arthritis Control group, highlighting the potential of AEMP in mitigating arthritis pathology.

4. Discussion

The evaluation of AEMP (Arthritis-Modifying Experimental Product) across various parameters has yielded significant insights into its therapeutic effects. In assessing paw edema volume, the treatment groups exhibited varying outcomes compared to the control group. While the arthritis control group demonstrated a significant delay in the therapeutic response, both the standard treatment and the higher AEMP dosage groups showed effects comparable to the control, suggesting that AEMP may possess therapeutic properties. Notably, all treatment groups exhibited reduced time compared to the negative control group, indicating a potential for AEMP to alleviate paw edema. However, comparisons among the treatment groups indicate that increasing the dosage may not necessarily enhance the therapeutic effect, suggesting a plateau in efficacy.

Further investigations into arthritic scores revealed that both doses of AEMP significantly reduced the severity of arthritis compared to the control, demonstrating a clear therapeutic potential. The higher dose of AEMP was found to be as effective as the standard treatment, indicating a dose-dependent response that warrants further exploration. The findings suggest that AEMP could serve as a viable intervention for arthritis, with implications for its clinical applications and effectiveness in managing arthritic conditions.

When examining biochemical markers, AEMP's impact on serum enzyme levels and oxidative stress indicators presented a nuanced picture. Increases in alkaline phosphatase (ALP) levels in the negative control group indicated potential adverse effects, while AEMP-treated groups showed significant decreases, suggesting a therapeutic effect. Additionally, AEMP treatment was associated with favorable changes in serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) levels, pointing to hepatoprotective effects. The findings indicate a potential restoration of liver function and antioxidant defenses, particularly at higher doses of AEMP.

Moreover, the assessment of oxidative stress markers such as superoxide dismutase (SOD) and reduced glutathione (GSH) levels underscored the antioxidative properties of AEMP. While the negative control exhibited decreased antioxidant defenses, treatment with AEMP resulted in significant increases in both SOD and GSH levels, indicating a restoration of antioxidant reserves. This effect was particularly pronounced at lower doses, suggesting that AEMP may

enhance the body's natural defense mechanisms against oxidative stress. Collectively, these findings highlight the multifaceted therapeutic potential of AEMP in managing arthritis and its associated biochemical and oxidative stress-related alterations, paving the way for future clinical investigations.

5. Conclusion

The experimental methodology involved inducing arthritis in male Wistar albino rats through the injection of Complete Freund's Adjuvant (CFA) into the left hind paw, followed by the division of the rats into five groups: a control group receiving saline, a negative control group treated with CFA alone, a standard group treated with dexamethasone, and two test groups receiving AEMP at low and high doses. After 28 days of treatment, assessments of various parameters, including paw edema volume, arthritic scores, and biomarker levels such as alkaline phosphatase (ALP), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), urea, lipid peroxidation (LPO), creatinine, catalase, superoxide dismutase (SOD), and reduced glutathione (GSH), revealed that both doses of AEMP exhibited significant anti-arthritic and anti-inflammatory effects. Specifically, AEMP-treated groups demonstrated decreased paw edema, reduced arthritic scores, and improved biomarker levels compared to the negative control group, with the higher dose showing comparable efficacy to dexamethasone. However, the presence of elevated ALP levels in some groups suggests potential hepatotoxicity, highlighting the need for further research to optimize dosage and mitigate adverse effects. The findings indicate that AEMP has considerable therapeutic potential for managing arthritis and related inflammatory conditions, warranting further investigation before clinical application.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest.

Statement of ethical approval

The protocols were approved by Institutional Animal Ethic Committee (IAEC) of Aadhibhagawan College of Pharmacy. Thiruvannamalai, Tamilnadu, India and Ethical approved number: 1696/po/a/13/CPCSEA.

Author Contribution

Vignesh M had principal responsibility for the conception and design of the study and drafting of the manuscript. Dr. P. Muralidaran was responsible for the revision of the manuscript for important intellectual content. P. Madhu Maya Devi, S. swetha, L. Gopi, K. Yuvaraj contributed towards reviewing of the literature. All authors agreed on the final version of the manuscript.

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