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Original Research Article

Evaluation of Antirheumatoid Arthritis Activity of Aqueous Methanol Extract of *Globba winitti* Rhizome

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ABSTRACT

Rheumatoid arthritis is an autoimmune disorder affecting about 0.35 – 1% of the world population. This study aimed to assess the effectiveness of the aqueous methanol extract of *Globba winitti* (AMEGW) in the treatment of Freund's Complete Adjuvant (FCA)-induced arthritis in rats. AMEGW was obtained by maceration in methanol, the acute toxicity of the extract was evaluated by single oral administration of 2000 mg/kg dose of AMEGW to Swiss mice. Arthritis was induced by intraplantar injection of FCA (0.1 mL) to the rats. The FCA-induced arthritic rats were treated with AMEGW at doses of 100, 200, and 400 mg/kg per oral,once daily for 28 days. The anti-arthritic effect of the extract was evaluated by measuring various parameters, including paw volume, joint diameter, pain threshold, thermal hyperalgesia (paw withdrawal latency), mechanical nociceptive threshold, and body weight. On day 28, rats were sacrificed, blood samples were collected for haematological, biochemical, and antioxidant analysis. Radiological and histopathological examination of ankle joints were also performed. AMEGW demonstrated anti-arthritic activity by reducing paw volume and joint diameter, accompanied by an increase in pain threshold, mechanical nociceptive threshold, paw withdrawal latency, and body weights of arthritic rats in a dose-dependent manner. AMEGW at doses of 400 and 200 mg/kg reversed the alterations in haematological, biochemical, and antioxidant parameters caused by FCA-induced arthritis. Furthermore, AMEGW effectively suppressed joint destruction, as evidenced by radiological and histopathological analysis. Therefore, *G. winitti* exhibits potential as a viable preventive or therapeutic alternative for the treatment of inflammatory conditions such as rheumatoid arthritis.

Keywords: Antiarthritic activity, *Globba winitti*, Pain threshold, Paw withdrawal, Aqueous methanol extract.

Introduction

Rheumatoid arthritis (RA) is a common disease that affect a large number of individuals worldwide.[1] It is an autoimmune disorder characterized by chronic inflammation due to synovial hyperplasia, which further progresses to massive irreversible bone destruction. RA is marked by damage to the joints due to an inflammatory response of synovial cells, resulting in complete loss of normal function and deformity. It affects approximately 1% of the overall global population.[2] Several medications, including biological agents such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1*β*) antagonists, as well as steroids and nonsteroidal anti-inflammatory drugs (NSAIDs) have been developed for the treatment of RA. These medications have demonstrated limited effectiveness in treating RA when used in combination with other conventional medical techniques. While these therapies show efficacy in treating the symptoms of acute RA, they are associated with several serious side effects, such as infections, gastrointestinal disorders, and increased cardiovascular risks.[3] For this reason, there is the need for the development of improved, potent, and effective agents with fewer side effects for the treatment of chronic, persistent RA.[4]

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Rats with FCA-induced arthritis are commonly used as models to study chronic systemic inflammation and show many similarities to human RA.[5] FCA-induced arthritis is commonly used to study the pharmacological and pathophysiological regulation of inflammatory processes as well as assess the antiarthritic effects of medications.[6] FCA-induced arthritis follows a two-phase temporal pattern; (i) it begins with an acute localized inflammatory response three to four days after induction, and (ii) after a relapsing-remitting phase beginning from the second week, a chronic systemic reaction is repeated for several months.[7] Although the exact cause of this biphasic pattern is unknown, it could be attributed to an initial stimulus from the FCA injection, which is known to cause a delayed hypersensitivity response.[8] *Globba* is a well-known genus of the Zingiberaceae family native to India, Malaysia, Indonesia, and China. More than a hundred species of this genus have been utilized historically to treat a variety of medical conditions,[9] such as postpartum complications, oral ulcers, eye injuries, infections, asthma, leucoderma, cough, food poisoning, pain, fever, angina, and stomach discomfort due to the presence of essential oils.[10] The present study aim to evaluate the theraupeutic potential of *Globba winitti* in reducing pain due to Rheumatoid arthritis.

Materials and Methods

Collection and identification of plant material

Globba winitti rhizomes were collected from the Chinnaraavigudem forest reserve in the Manuguru Mandal of the Bhadradri district in Telangana state, India in December 2019. The specimens were identified and verified by K. Madhava Chetty, a plant taxonomist from Sri Venkateswara University" in Tirupathi, Andhra Pradesh, and preserved as herbarium specimens. The voucher number 0912 was assigned to the specimens.

Experimental animals

Female Wistar rats weighing between 180 and 220 g and Swiss albino mice weighing between 25 and 30 g were obtained from the National Toxicology Centre in Pune, India. The animals were kept at room temperature (25 ± 1ºC) under a controlled relative humidity of 45 – 55%, with a 12 h light-12 h dark cycle.

Ethical approval

The protocol was approved by the Institutional Animal Ethics Committee (IAEC)[11] which adopt the rules, regulations, and guidelines set forth by the PBCSEA, India. Ethical approval reference number ASPEN/11/2020 was issued.

Preparation of extract

Globba winitti rhizomes were dried under a shad, the dried rhizomes were pulverized into a fine powder.[12-14] A 100 g quantity of the dried powder was extracted by maceration in aqueous methanol of volume 250 ml at room temperature with intermittent shaking. The extract was filtered, and the filtrate was concentrated to obtain an extract yield of 6.1%. The extract was dissolved in a 0.1% v/v solution of Tween-80 to form a suspension, which was used for the animal experiment.[15]

Acute oral toxicity study

Acute oral toxicity test was performed using selected healthy female Swiss mice following the OECD guideline 425. The mice were fasted overnight, but allowed free access to drinking water. A dose of 2000 mg/kg body weight of *G. winitti* rhizome aqueous methanol extract (AMEGW) was administered to the mice via the oral route. The mice were observed for signs of toxicity for the first two hours, and thereafter monitored for changes in autonomic and behavioral traits, as well as mortality for 48 hours.[16]

Freund's complete adjuvant-induced arthritis

Arthritis was induced by the injection of 0.1 mL Freund's complete adjuvant (solution of 1 mg *Mycobacterium tuberculosis* (Strain H37Ra, ATCC-25177) per milliliter) to the left hindpaw footpads of female Wistar rats. Prior to induction of arthritis, the rats were anesthetized by ether inhalation to fascilitate the injection of the adjuvant, which is usually difficult to inject due to its viscous nature.[17] The rats were divided into six groups of six animals each. Group I is the healthy control group which consist of non induced healthy animals, Group II is the arthritic control which is made up of FCA-induced arthritic rats without extract treatment, Group III represent the positive control, which is made up of arthritic rats administered an oral dose of diclofenac (5 mg/kg), Group IV is made up of arthritic rats administered an oral dose of AMEGW (100 mg/kg), Group V is made up of arthritic rats administered an oral dose of AMEGW (200 mg/kg), and Group VI is made up of arthritic rats administered an oral dose of AMEGW (400 mg/kg). The test sample (AMEGW), as well as the positive control (diclofenac) were administered once daily for 28 days. Several factors, including tactile allodynia, body weight, joint diameter, paw volume, and thermal hyperalgesia were assessed during the study period to evaluate the efficacy of AMEGW in treating arthritis.[18] After the 28th day treatment, the animals were placed under ether anesthesia, and blood samples were collected from the retro-orbital plexus. The blood sample was used for analysis of hematological and biochemical parameters.

Determination of paw volume

The paw volume was measured using a Plethysmometer (UGO Basile, Italy) on day 0 before FCA injection as well as on days 1, 4, 8, 12, 16, 20, 24, and 28.[19] The change in paw volume was calculated by subtracting the initial paw volume from the final paw volume.

Determination of joint diameter

The diameter of the joint was measured using a digital vernier caliper (Mitutoyo, Japan) on day zero prior to FCA injections as well as on days 1, 4, 8, 12, 16, 20, 24, and 28.[20] The change in joint diameter was calculated by subtracting the initial joint diameter from the final joint diameter.

Determination of pain threshold

The pain threshold was measured on day zero before FCA injections and subsequently on days 1, 4, 8, 12, 16, 20, 24, and 28 using the Randall-Selitto analgesiometer (UGO Basile, Italy). Increasing force was applied to the hind paw, which had been positioned between the blunt end and the flat surface. The threshold for pressure was 450 grams. The pain threshold was assessed using the rat's response when it removed its hind paw from the analgesiometer.[21]

Determination of thermal hyperalgesia (Paw withdrawal latency)

Using the radiant heat apparatus (UGO Basile, Italy), the thermal sensitivity of the animal paw was assessed on multiple occasions: the day before the FCA injections, day 0, days 1, 4, 8, 12, 16, 20, 24, and 28. Upon placing the "paw on the heat radiator, the intensity of the infrared lamp was adjusted to 40. A 15-second latency period was allowed to minimize any possible harm to the tissue.[22]

Determination of mechanical nociceptive threshold

The mechanical nociceptive threshold was determined by applying a calibrated fine filaments (Von Frey hairs, Almemo, Germany) to the foot paw and the paw withdrawal response was observed.[23,24] The rats were allowed to adapt in a Perspex box for a duration of 10 minutes, after which Von Frey hairs with weights ranging from 0.6 to 12.6 grams were positioned on the left hind paw's flat surface. Every individual hair on each paw experienced three stimuli consecutively within a time frame of 2-3 seconds. The threshold was determined by taking the least amount of Von Frey's hair necessary for generating a withdrawal response after three consecutive applications. Paw lifting was taken as a positive reaction.[25]

Measurement of body weight

The body weight of the rats were measured on day zero prior to FCA injection, as well as on days 1, 4, 8, 12, 16, 20, 24, and 28.[26]

Radiological analysis of ankle joints

On the 28th day, the rats were anesthesized, and X-rays of the hind paws injected with FCA was taken using a German AGFA CR 30-X X-ray machine. Radiography was performed on the hind paws using a peak voltage of 55kV and a current of 50mA. The animals were exposed for period of 5 seconds.

Measurement of hematological and serum parameters

Hematological parameters, including haemoglobin (Hb) concentration, platelets (PLT) count, red blood cell (RBC) count, and white blood cell (WBC) count were measured on the 28th day using a standard laboratory technique.[27] Serum levels of C-reactive protein (CRP), and Rheumatoid factor (RF) were also assessed.

Measurement of biochemical parameters

The serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and total protein levels were determined according to standard procedures.

Measurement of antioxidant parameters

On day 28, the rats were sacrificed by cervical dislocation, and their livers were removed and washed with a cold saline solution. The liver was homogenized with a 0.1 M tris-HCl buffer (pH 7.4). the levels of antioxidant enzymes [superoxide dismutase (SOD), and glutathione (GSH)], as well as malondialdehyde (MDA) were measured in the homogenate. The protocols described by Misera and Fridocich (1972),[28] Morgon *et al*. (1979),[29] and Slater and Sawyer (1971)[30] were followed in performing the SOD, GSH, and MDA assays, respectively.

Histopathological analysis of ankle joints

After 28 days, the ankle joints were separated from the hind paw and left in a 10% buffered formalin solution for a full day. Subsequently, they were subjected to decalcification using a 5% solution of formic acid, and subsequently prepared for paraffin embedding. Tissue sections (5 µm thick) were stained with hematoxylin-eosin, they were examined under a light microscope at ten different magnifications to determine whether inflammatory cells, synovial hyperplasia, pannus formation, or destruction of the joint space were present.[31]

Measurement of spleen and thymus weight

On the 28th day, the rats' spleens and thymus were surgically extracted,

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and their weights were measured.[32]

Statistical analysis

Data were presented as mean ± SEM. Statistical analysis was done using GraphPad 5.0 (GraphPad, San Diego, USA) statistical package. Data were subjected to one way analysis of variance (ANOVA), followed by Dunnett's test or Bonferroni test. Statistical significant difference was established at $P < 0.05$.

Results and Discussion

Acute toxicity of AMEGW

Upon oral administration of AMEGW at a dose of 2000 mg/kg body weight, no sign of toxicity or mortality were observed. This suggests that the extract is not toxic at a dose of 2000 mg/kg. Consequently, 3 doses (100, 200, and 400 mg/kg b.w) were chosen for the pharmacological investigations.

Antirheumatoid arthritis activity of AMEGW

RA is a chronic inflammatory disease that affects approximately 16% of individuals in the developed nations.[33] Both human arthritis and adjuvant-induced arthritis in rats exhibit similar clinical manifestations, such as limb edema, infiltration of inflammatory cells, proliferative synovitis, and degradation of bone and cartilage structure. Because of the similar pathological features, the adjuvant-induced arthritic rat is often used as a model for RA in order to assess the effectiveness of anti-inflammatory medications.[34] Alternative RA treatments are becoming increasingly popular. Many medicinal plants can potentially reduce the incidence of rheumatoid arthritis symptoms, with effects resembling those of currently marketed conventional pharmaceuticals.[35] The current investigation discovered that the administration of AMEGW at doses of 400 and 200 mg/kg demonstrated a potential therapeutic effect in rheumatoid arthritis, as evidenced by improvements in all inflammatory parameters.

Effect of AMEGW on paw volume

All rats treated with FCA showed a significant ($P < 0.001$) increase in paw volume compared to the normal control group. Beginning from the 20th day, there was a significant ($P < 0.01$) decrease in paw volume in the AMEGW-treated rats compared to the arthritic control group. On day 28, AMEGW administration at a dose of 100 mg/kg resulted in a significant ($P<0.01$) decrease in paw volume. For the positive control group (diclofenac 5 mg/kg), there was a significant ($P < 0.01$) reduction in paw volume beginning from the 16th day. On the other hand, rats treated with 400 mg/kg and 200 mg/kg AMEGW showed a significant reduction in paw volume (1.42 ± 0.18 mm, and 2.13 ± 0.07 mm, respectively) on day 28 compared to the arthritic control group with average paw diameter of 3.81 ± 0.10 mm (Figure 1).

Effect of AMEGW on joint diameter

FCA treatment resulted in a significant ($P < 0.001$) increase in joint diameter compared to the healthy control group. However, AMEGW treatment at doses of 200 and 400 mg/kg resulted in a significant decrease in joint diameter starting from day 20 compared to the arthritic control group. On day 28, the average joint diameter were 2.12 ± 0.06 mm, and 1.39 ± 0.19 mm for the 200 mg/kg, and 400 mg/kg dose of AMEGW, respectively, whereas the average joint diameter for the arthritic control group was 3.37 ± 0.14 mm (Figure 2).

The reduction in paw volume and joint diameter by AMRGW treatment compared to the arthritic control group suggests a significant reduction in inflammation. The results of the current investigation showed that rats administered FCA exhibited increased paw size and joint diameter, which is an indication of stiff ankles.

Effect of AMEGW on pain threshold

The rats administered FCA experienced a gradual decrease in the pain threshold of their paw until day 12. Administration of AMEGW at 400 mg/kg, and 200 mg/kg doses resulted in a notable and statistically significant ($P < 0.001$) increase in pain threshold on days 20, and 24, respectively.

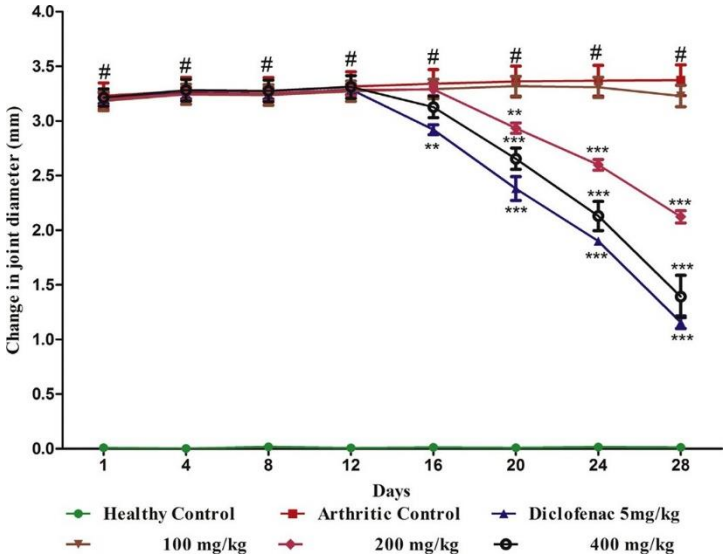

Figure 1: Effect of AMEGW on paw volume in FCA-induced arthritis in rats. Values represent the mean ± standard error of mean (SEM), n = 6. ** P<0.01 and *** P<0.001 compared to the arthritic control group, # P < 0.001 compared to the Healthy control group.

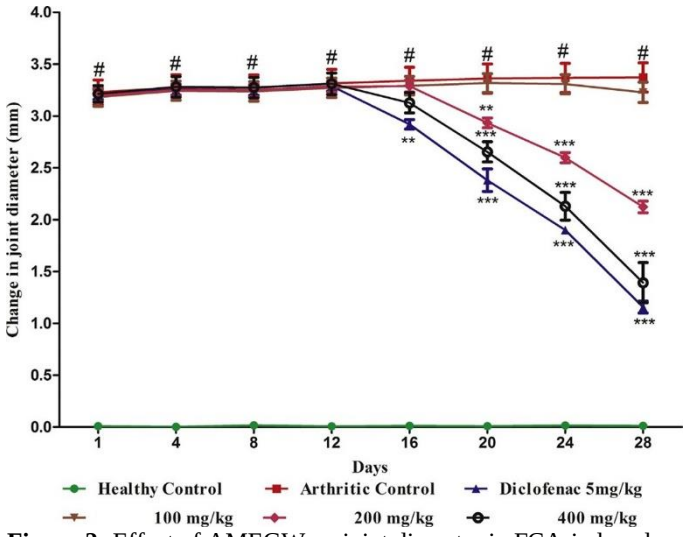

Figure 2: Effect of AMEGW on joint diameter in FCA-induced arthritis in rats. Values represent the mean ± standard error of mean (SEM), n = 6. ** P<0.01 and *** P<0.001 compared to the arthritic control group, # P < 0.001 compared to the healthy control group.

Although, AMEGW at a dose of 100 mg/kg had a lower efficacy compared to the higher doses, albeit it still increased the pain threshold on day 28. On the other hand, administration of diclofenac at a dose of 5 mg/kg led to a significant ($P<0.001$) increase in pain threshold, beginning from day 20. As illustrated in Figure 3, on day 28, the pain threshold of the AMEGW (400 mg/kg) group was 250 ± 15.2 g, while AMEGW at 200 mg/kg had pain threshold of 224 ± 4.7 g, which were significantly higher than that of the arthritic control group with a pain threshold of 145 ± 7.9 g.

Effect of AMEGW on thermal hyperalgesia (Paw withdrawal latency)

Rats in the FCA-treated group showed considerable and statistically significant ($P<0.001$) lower paw withdrawal latency compared to the

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healthy control group. However, administration of AMEGW at doses of 400 mg/kg and 200 mg/kg led to a significant (P<0.01) increase in paw withdrawal latency on days 20 and 24, respectively. Nevertheless, the effect of AMEGW administration at a dose of 100 mg/kg on day 28 was negligible, albeit resulting in a slight increase in paw withdrawal latency (P<0.05). similarly, administration of diclofenac at a dose of 5 mg/kg caused a significant (P<0.001) increase in paw withdrawal latency, beginning from day 20. As shown in Figure 4, on day 28, the paw withdrawal latency in the AMEGW groups at 400 mg/kg (7.32 ± 0.25 sec) and 200 mg/kg (6.17 ± 0.36 sec) were significantly higher than that of the arthritic control group (2.90 ± 0.18 sec).

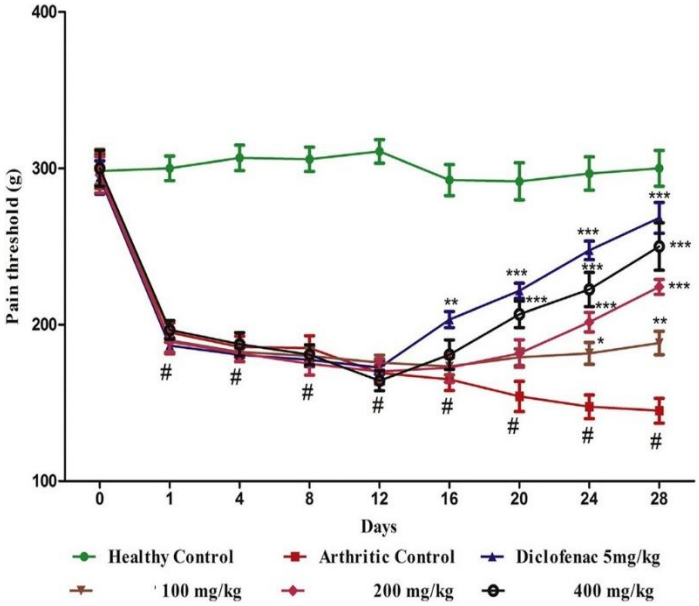

Figure 3: Effect of AMEGW on pain threshold in FCA-induced arthritis in rats. Values represent the mean ± standard error of mean (SEM), n = 6. * P<0.05, ** P<0.01, and *** P<0.001 compared to the arthritic control group, # P < 0.001 compared to the healthy control group

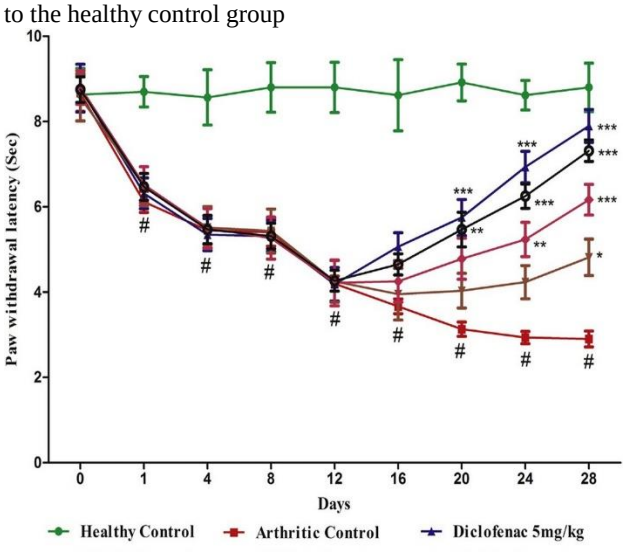

Figure 4: Effect of AMEGW on thermal hyperalgesia (paw withdrawal latency) in FCA-induced arthritis in rats. Values represent the mean ± standard error of mean (SEM), n = 6. * P<0.05, ** P<0.01, and *** P<0.001 compared to the arthritic control group, # P < 0.001 compared to the healthy control group

Effect of AMEGW on mechanical nociceptive threshold

As shown in Figure 5, the lowest mechanical nociceptive threshold was recorded for all the treatment groups on day 12. The administration of AMEGW at doses of 400 mg/kg, and 200 mg/kg resulted in a significant (P<0.001) improvement in the mechanical nociceptive threshold beginning from day 20 compared to the arthritic control group. However, the group that received AMEGW at 100 mg/kg showed only a slight improvement in the mechanical nociceptive threshold, but a significant (P<0.01) improvement was observed on day 28. For the diclofenac (5 mg/kg) group, a significant (P<0.001) improvement in mechanical nociceptive threshold was evident as from the 16th day. The mechanical nociceptive threshold were 59.82 ± 1.01 g, and 52.58 ± 1.76 g for the 400 mg/kg, and 200 mg/kg AMEGW, respectively, while that arthritic control control group was 24.97 ± 0.98 g.

Effect of AMEGW on body weight of rats

The rats in the arthritic control group exhibited a reduction in body weight compared to the groups that received treatment with AMEGW and diclofenac. On Day 28, the body weight of rats in the AMEGW group 400 mg/kg, and 200 mg/kg were 209 ± 4.10 g and 201 ± 5.08 g, respectively, and these were significantly higher than the body weights of those in the arthritic control group, which was 168 ± 3.50 g. The findings showed that on day 28, the administration of AMEGW at doses of 400 and 200 mg/kg increased body weight by 23.89%, and 19.15%, respectively, while the administration of diclofenac at a dose of 5 mg/kg resulted in a 25.37% increase in body weight when compared to the arthritic control group (Figure 6). The reduction in body weight during inflammation is a result of decreased absorption of nutrients via the intestine.[36] Consequently, the increase in body weight of rats treated with AMEGW can be attributed to improved nutrient absorption in the rat's intestines.

Effect of AMEGW on haematological and serum parameters

Studies have shown that arthritis can cause a moderate elevation in the number of WBC. The IL-1β is thought to be responsible for this increase, as it promotes the production of colony-stimulating factors. Injection of FCA in rats resulted in an increased platelet and WBC counts, and a decreased RBC count and hemoglobin levels. The administration of AMEGW resulted in a dose-dependent changes in these parameters (Table I). The administration of AMEGW at doses of 400 and 200 mg/kg effectively decreased the abnormally high levels of serum CRP and RF in FCA-induced arthritis (Table I). The reduction in hemoglobin count in arthritis is attributed to a diminished bone marrow response to erythropoietin, decreased levels of erythropoietin, and premature destruction of RBCs.[37] The current investigation illustrated that the application of AMEGW led to a notable decrease in white blood cell count and an elevation in hemoglobin levels. Furthermore, both AMEGW and diclofenac effectively reversed other notable haematological alterations, including the reduction in red blood cell count and the elevation in platelet count. AMEGW treatment considerably lowered the levels of CRP and RF. RA is a condition marked by a significant increase in the incidence of distal interphalangeal joint (DIPJ) arthritis. RF can be used as a marker for RA.[38] An enduringly elevated serum concentration of CRP is widely recognized as a strong indicator of RA.[39]

Effect of AMEGW on biochemical parameters

The current study found that the administration of FCA led to a notable increase in serum AST, ALT, and ALP, along with a corresponding decrease in total protein. Measuring the serum levels of ALT, AST, and ALP is a reliable and straightforward way to evaluate the ability of a drug to treat arthritis. Elevated levels of aminotransferases and alkaline phosphatase are observed in arthritic rats, as these enzymes are reliable markers of liver and kidney dysfunction, which is also a characteristic of adjuvant-induced arthritis. The serum levels AST and ALT have been shown to notably influence the synthesis of chemical mediators known as bradykinins in the inflammatory mechanism.[40] As presented in Table 2, rats in the arthritic control group showed a notable rise in serum levels of AST, ALT, and ALP (P<0.001), as well as a significant decrease in total protein level, which was due to FCA-injection.

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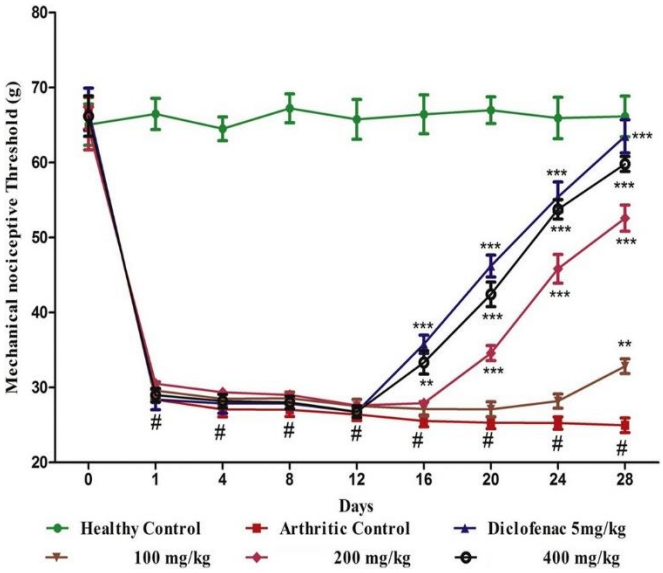

Figure 5: Effect of AMEGW on mechanical nociceptive threshold in FCA-induced arthritis in rats. Values represent the mean ± standard error of mean (SEM), n = 6. ** P<0.01, and *** P<0.001 compared to the arthritic control group, # P < 0.001 compared to the healthy control group

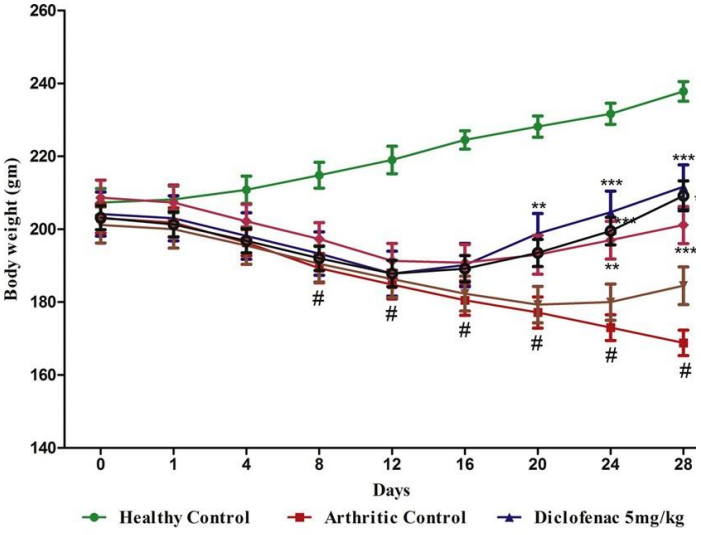

Figure 6: Effect of AMEGW on body weight of FCA-induced arthritic rats. Values represent the mean ± standard error of mean (SEM), n = 6. ** P<0.01, and *** P<0.001 compared to the arthritic control group, # P < 0.001 compared to the healthy control group

However, the administration of AMEGW and diclofenac altered the serum levels of these enzymes in a dose-dependent manner. It is important to state the administration of AMEGW at a dose of 400 mg/kg led to a significant (P<0.001) reduction in AST, ALT, and ALP levels, while simultaneously increasing the total protein level. This finding validates the antiarthritic activity of the extract.

Effect of AMEGW on antioxidant parameters

Free radicals are essential contributors to the progression of various diseases, like arthritis, inflammation, cancer, and liver disorders.[41] Lipid peroxidation is an essential process that plays a significant role in the observed damage in rheumatoid arthritis. This damage is usually evaluated by analyzing tissue MDA levels. Results of the present study showed that malondialdehyde (MDA) concentrations in the liver of the arthritic rats were significantly (P<0.001) higher than those in the healthy rats. The elevated levels of MDA noted in the arthritic control group suggest that the damage results from the presence of free radicals.[42] Treatment with AMEGW at 200 and 400 mg/kg doses led to a notable reduction in MDA levels (Table 3).

GSH functions as an intracellular reducing agent in oxidation-reduction reactions, playing a vital role in safeguarding against harm caused by reactive oxygen species (ROS) and organic peroxides. The overconsumption of GSH in order to protect against oxidative damage may be the cause of the decreased GSH levels in the liver of arthritic rats.[43] Arthritis causes the production of oxygen free radicals, which in turn reduces the levels of GSH and SOD due to their consumption during metabolic breakdown and oxidative stress. This decline is apparent through the reduced concentrations of GSH and SOD observed in the arthritic control group. Administration of AMEGW (400 and 200 mg/kg) significantly ((P <0.001) restored the depleted levels of GSH and SOD in the rats (Table 3). This restoration is likely attributed to AMEGW's ability to scavenge free radicals effectively.

Effect of AMEGW on ankle joints following radiological examination

The radiological analysis examined the occurrence of bone destruction, a common feature of adjuvant-jnduced arthritis.[44] Radiological examination of the ankle joints of FCA-induced arthritic rats revealed a reduced joint space in the inter-tarsal joints, a widespread soft tissue inflammation, increased bone cyst' size, and extensive erosion. The rat paw X-ray analyses showed that treatment with AMEGW (400 mg/kg) and diclofenac (5 mg/kg) resulted in a great and significant reduction in bone destruction. This indicated that AMEGW is effective in preventing the joint changes caused by arthritis. On the other hand, administration of AMEGW at 200 mg/kg dose led to a moderate reduction in bone destruction. However, no noticeable effect was observed when AMEGW was administered at a dose of 100 mg/kg (Figure 7).

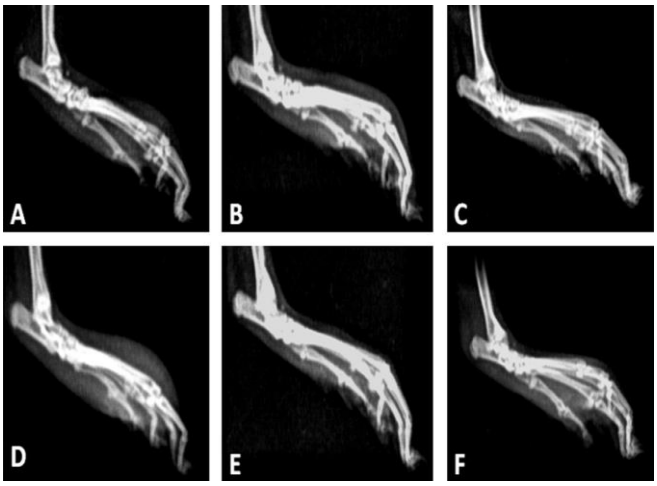

Figure 7: Photomicrograph of ankle joint on radiographic analysis **(A):** Healthy control **(B):** Arthritic control **(C):** Diclofenac 5 mg/kg treated group **(D):** AMEGW 100 mg/kg treated group **(E):** AMEGW 200 mg/kg treated group **(F):** AMEGW 400 mg/kg treated group

Histopathological features of rats' ankle joints following AMEGW treatment

The histopathological analysis of the ankle joint provides clear evidence that treatment with AMEGW (at 400 and 200 mg/kg doses) can effectively manage connective tissue inflammation. The histopathological examination of the ankle joint of healthy female Wistar rats showed no evidence of inflammation. The synovial lining was intact, and no bone necrosis was observed (Figure 8). The number of inflammatory cells, bone necrosis, chronic inflammation, and disruption of the synovial lining were all significantly increased in the rats treated with FCA.

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Table 1: Effect of AMEGW on Haemotological *and Serum* Parameters in FCA-Induced Arthritis in Rats

Treatment Group	RBC (10^6cells/mm^3)	WBC (10^3cells/mm^3)	Hb (g/dL)	Platelets (10^3cells/mm^3)	CRP (mg/L)	RF (IU/mL)
Normal control	6.80 ± 0.26	7.30 ± 0.33	14.30 ± 0.22	903 ± 20	1.65 ± 0.14	-
Arthritic control	3.50 ± 0.20	14.20 ± 0.25	9.40 ± 0.26	1734 ± 40	7.05 ± 0.26	57 ± 1.20
Diclofenac (5 mg/kg)	6.10 ± 0.26	8.40 ± 0.25	14.10 ± 0.35	1091 ± 49	2.88 ± 0.17	34 ± 0.91
AMEGW (100 mg/kg)	3.10 ± 0.06	11.40 ± 0.63	11.80 ± 0.40	1524 ± 56	6.30 ± 0.31	51 ±1.10
AMEGW (200 mg/kg)	5.50 ± 0.33	8.50 ± 0.45	12.66 ± 0.25	1455 ± 45	4.12 ± 0.32	45 ±1.30
AMEGW (400 mg/kg)	5.70 ± 0.34	8.10 ± 0.23	13.40 ± 0.27	1350 ± 47	4.14 ± 0.42	41 ± 0.81

Values are mean ± standard error of mean (SEM), n = 6. AMEGW = Aqueous methanolic extract of *Globba winitti* rhizome, FCA = Freund's complete adjuvant, RBC = Red blood cells, WBC = White blood cells, Hb = Haemoglobin, CRP = C-reactive protein, RF = Rheumatoid factor.

Table 2: Effect of AMEGW on Biochemical parameters inFCA-Induced Arthritis in Rats

Treatment Group	AST (U/L)	ALT (U/L)	ALP (U/L)	Total protein (g/dL)
Healthy control	42 ± 2.30	42 ± 1.70	73 ± 3.30	6.60 ± 0.06
Arthritic control	127 ± 4.40	173 ± 4.80	475 ± 16.0	5.10 ± 0.04
Diclofenac (5 mg/kg)	59 ± 3.30	57 ± 2.00	127 ± 6.70	6.40 ± 0.04
AMEGW (100 mg/kg)	120 ± 0.06	159 ± 2.21	446 ± 19.40	5.10 ± 0.04
AMEGW (200 mg/kg)	90 ± 0.33	119 ± 3.30	335 ± 12.25	5.40 ± 0.04
AMEGW (400 mg/kg)	70 ± 0.34	73 ± 2.20	197 ± 6.27	6.40 ± 0.04

Values are mean ± standard error of mean (SEM), n = 6. AMEGW = Aqueous methanolic extract of *Globba winitti* rhizome, FCA = Freund's complete adjuvant, AST = Aspartate aminotransferase, ALT = Alanine aminotransferase, ALP = Alkaline phosphatase.

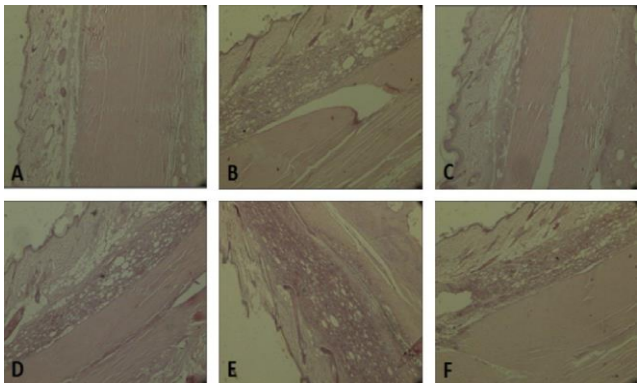

Figure 8: Photomicrograph of ankle joint tissues following histopathological examination of the ankle joints. Tissue were stained with hematoxylin and eosin (H&E) dye **(A):** Healthy control **(B):** Arthritic control **(C):** Arthritic rats treated with 5 mg/kg diclofenac. **(D):** Arthritic rats treated with 100 mg/kg dose of AMEGW **(E):** Arthritic rats treated with 200 mg/kg dose of AMEGW **(F):** Arthritic rats treated with 400 mg/kg dose of AMEGW

In contrast to the observed pathological alterations in the arthritis group, the rats that were administered AMEGW (200 and 400 mg/kg) and diclofenac (5 mg/kg) exhibited a significant defense against bone necrosis, accompanied by a decreased presence of inflammatory cells. In contast, rats administered 100 mg/kg of AMEGW orally showed signs of bone necrosis, disruption of the synovial lining, and increased inflammatory cell infiltration (Figure 8).

Effect of AMEGW on spleen and thymus weights

The weight of the spleen and thymus decreased significantly after AMEGW administration at doses of 200 and 400 mg/kg compared to the arthritic control group. It has been shown that immunostimulatory effect and the decrease in spleen weight are related.[45]

On the final day of the study (day 28), the weights of spleen (0.75 ± 0.02 g) and the thymus (0.15 ± 0.01 g) in the AMEGW (400 mg/kg) treated group was were significantly lower than those of the spleen (0.91 ± 0.03 g) and thymus (0.21 ± 0.01 g) in the arthritic control group. The observed decrease in the weights of these organs due to AMEGW administration was statistically significant ($P < 0.001$). Similarly, the oral administration of AMEGW at a dose of 200 mg/kg led to a significant ($P<0.05$) reduction in the weights of the spleen (0.82 ± 0.02 g) and thymus (0.18 ± 0.01 g). However, when AMEGW was given orally at a dose of 100 mg/kg, it resulted in no significantly impact on the weights of the spleen

and thymus. Just as the AMEGW 200 and 400 mg/kg doses, the positive control (diclofenac 5 mg/kg) resulted in a significant (P<0.001) reduction in the weights of the spleen (0.65 ± 0.02 g) and thymus (0.12 ± 0.01 g) compared to the arthritic control group.

Table 3: Effect of AMEGW on Antioxidant parameters in FCA-Induced Arthritis in Rats

Treatment Group	SOD (Units/mg protein)	GSH (µg/mg protein)	MDA (nmol/mg protein)
Normal control	4.80 ± 0.06	74.00 ± 2.00	2.00 ± 0.03
Arthritic control	2.50 ± 0.03	45.00 ± 1.80	3.60 ± 0.06
Diclofenac (5 mg/kg)	3.70 ± 0.03	64.00 ± 2.20	2.80 ± 0.04
AMEGW (100 mg/kg)	2.40 ± 0.06	51.00 ± 2.06	3.80 ± 0.04
AMEGW (200 mg/kg)	3.33 ± 0.05	59.00 ± 1.06	3.30 ± 0.02
AMEGW (400 mg/kg)	4.80 ± 0.06	63.00 ± 2.90	3.10 ± 0.04

Values are mean ± standard error of mean (SEM), n = 6. AMEGW = Aqueous methanolic extract of *Globba winitti* rhizome, FCA = Freund's complete adjuvant, SOD = Superoxide dismutase, GSH = Glutathione, MDA = Malonaldehyde.

Conclusion

The findings from the present study have shown that AMEGW has antiarthritic activity when given at doses of 200 and 400 mg/kg due to it analgesic and anti-inflammatory effects on a number of measured parameters in arthritic rats. AMEGW affects different parameters including hematological, biochemical, and antioxidant parameters, as well as radiological and histopathological features. These findings suppots the use of *Globba winitti* rhizome as a traditional herbal remedy for inflammatory conditions like rheumatoid arthritis.

Conflict of interest

The authors declare no conflicts of interest.

Authors Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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