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Ethanol Extract of *Caryota urens* Lour Fruits Alleviates Oxidative Stress in a Murine Model of Rheumatoid Arthritis Induced by Freund's Complete Adjuvant

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ABSTRACT

Traditional knowledge suggests that *Caryota urens* L. exhibits antioxidant properties. This study aims to assess the anti-inflammatory and antioxidant effects of ethanol extract from *C. urens* fruit (ECUF) on a mouse model of CFA-induced arthritis. The efficacy of ECUF was evaluated through parameters including body weight, body and paw temperature, levels of malondialdehyde (MDA), glutathione (GSH), total protein (TP), glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT). Results revealed significant inflammation induced by CFA injection, evidenced by increased body temperature and paw temperature ($p < 0.05$), accompanied by decreased body weight ($p < 0.05$). Treatment with ECUF significantly reduced body temperature (37.13 ± 0.03 °C) and paw temperature (27.22 ± 0.03 °C) ($p < 0.05$), while increasing body weight (35.21 ± 1.52 g) ($p < 0.05$). Arthritic mice exhibited elevated MDA levels in joint tissues, livers, kidneys, and spleens ($p < 0.05$), indicating increased oxidative stress. However, treatment with ECUF effectively reduced MDA levels (7.33 ± 0.61, 30.45 ± 5.47, 5.01 ± 0.41, 3.73 ± 0.21 nM/mg protein, respectively) ($p < 0.05$). Administration of ECUF also led to dose-dependent increases in GSH, TP, GPx, SOD, and CAT levels in joint tissues, liver, kidney, and spleen ($p < 0.05$). The maximal therapeutic effect of ECUF was observed at a dose of 300 mg/kg, comparable to Mobic ($p > 0.05$). Overall, these findings underscore the potential of ECUF as a therapeutic agent for arthritis due to its antioxidant properties and ability to reduce oxidative stress.

***Keywords*:** Antioxidant, *Caryota urens* Lour, Freund's complete adjuvant, Oxidative stress, Therapeutic potential.

Introduction

Rheumatoid arthritis (RA) stands out as one of the most destructive forms of inflammatory joint disease. It is a chronic autoimmune condition where non-purulent synovial inflammation contributes to the degradation of cartilage and bone, leading to inflammation in multiple joints. RA affects joint pairs, such as both hands or feet and can extend to smaller joints in the wrists and hands. Various joints may progressively deform over time, including knees, elbows, shoulders, ankles, and metatarsophalangeal joints. Additionally, other organs like the skin, eyes, and lungs may also be affected, resulting in complications such as neurological disorders, anemia, fatigue, and cardiac issues.[1] The etiology of RA is intricate, involving the simultaneous occurrence of various factors, including innate immune dysregulation, modulation of the cytokine network, activation of osteoclasts and chondrocytes, and stimulation of dendritic cells leading to the production of matrix metalloproteinases and pro-inflammatory cytokines, causing joint inflammation and damage.[2]
Freund's complete adjuvant (FCA) contains heat-killed *Mycobacterium tuberculosis*, which stimulates the immune system and induces a robust inflammatory response, mimicking the immune processes observed in RA. Therefore, they are commonly employed in research to establish experimental models of rheumatoid arthritis (RA) in animals.[3]

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The current study utilized FCA to induce RA-like symptoms in mice. By employing FCA to induce arthritis, the study aimed to replicate the intricate inflammatory and immune processes observed in RA, thereby providing valuable insights into potential therapeutic strategies. Subsequently, the study investigated the potential therapeutic effects of ethanol extract derived from *C. urens* fruits (ECUF) on oxidative stress and inflammation associated with this RA model.
Oxidative stress is often associated with elevating intracellular reactive oxygen species (ROS) levels, leading to damage across various biological molecules. ROS is generated during cellular respiration and is routinely eliminated to maintain cellular homeostasis and support physiological functions. In many diseases, including rheumatoid arthritis (RA), ROS plays a crucial role in the pathogenic mechanism. They also serve as central players in key pathways such as TNF-α and nuclear factor-kappa B (NF-κB), which are central to inflammatory reactions. The inflammatory state associated with RA often stems from alterations in signaling pathways, resulting in increased concentrations of inflammatory cytokines, lipid peroxides, and ROS. The body's natural defense mechanisms against oxidative stress involve antioxidant enzymes like catalase (CAT), Superoxide dismutase (SOD), and glutathione peroxidase (GPx), as well as non-enzyme antioxidants such as glutathione (GSH).[4] Cells have evolved a series of antioxidant defense systems to protect themselves from harmful ROS and free radicals. Imbalances in ROS production or elimination are closely linked to various diseases, including neurodegenerative disorders, cardiovascular diseases, and liver diseases. Oxidative stress often leads to cellular damage, especially to nucleic acids, proteins, and lipids, ultimately resulting in cell death.[4] Given the adverse effects and toxicity associated with the use of anti-inflammatory drugs, there is an increasing focus on exploring alternative medications based on natural products, deemed safer and more effective due to their antioxidant activities.[1]
Caryota urens L. (*C. urens*), a palm species belonging to the Aceraceae family, is widely distributed in several Asian countries such as India, Sri Lanka, Malaysia, Indonesia, and the Philippines. This plant

predominantly thrives in evergreen, semi-evergreen, and moist deciduous forests. In Sri Lanka, the young flowers of *C. urens* are traditionally harvested for sweet phloem sap, utilized in the production of sweeteners (honey and jaggery), and fermented beverages (toddy).[6] Folk wisdom attributes health-enhancing properties to both the sap and resin of *C. urens*. Traditional health benefits of *C. urens* resin, including antioxidant capabilities, blood sugar reduction, and various other properties, are primarily regulated through antioxidant effects such as free radical elimination, electron reduction, and metal ion excretion.[7] Extracts from *C. urens* leaves, fruits, and fruit peels have demonstrated potent antioxidant and antibacterial activities against pathogens like *Escherichia coli, Vibrio cholerae, Salmonella typhii, Staphylococcus aureus,* and *Shigella flexneri*.[8] Fermented *C. urens* sap exhibits diverse biological activities, including in vivo antioxidant effects, in vitro antioxidant properties, anti-obesity effects, diabetes treatment, and liver protection.[9] This underscores the diversity and wide-ranging applications of *C. urens* in the fields of health and medicine. Despite numerous studies on the properties of *C. urens*, there is currently no report focusing on its ability to counteract oxidative stress. Therefore, the purpose of this study is to evaluate the antioxidant capacity of ethanol extract from the fruit of *Caryota urens* L. and elucidate its potential to alleviate oxidative stress in a low-grade inflammatory joint disease mouse model induced by Freund's complete adjuvant. This research may open avenues for harnessing the medical potential of *C. urens* and encourage further studies on its applications in the treatment and prevention of oxidative stress-related disorders.

Materials and Methods

Reagents, chemicals, and equipment

The Freund's Complete Adjuvant (CFA), comprising 10 mL per unit (with each 1 mL containing 1 mg of *Mycobacterium tuberculosis*, sterilized and desiccated, along with 0.85 mL of paraffin oil and 0.15 mL of mannide monooleate), was supplied by Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). The ELISA kit, employed for quantifying inflammatory mediators, and the apparatus for analyzing oxidative stress parameters were procured from RayBiotech, Inc. (Norcross, GA) and imported through Nam Khoa Company Limited (Vietnam). All test reagents and chemicals utilized ensured high-quality analysis and purity, originating from Sigma (St. Louis, MO).

Collection of plant material

Caryota urens L. fruits were harvested in Atiêng commune, Tây Giang district, Quảng Nam province (Coordinates: 15°53'1"N 107°29'37"E), in November 2023. The collected samples underwent meticulous cleaning under aseptic conditions using distilled water 3-4 times to eliminate impurities. Subsequently, they were finely chopped and sun-dried for three consecutive days, followed by further dehydration until a constant mass was achieved at 60°C in a Memmert drying cabinet (Germany). The final dried fruits were pulverized into a fine powder using the MN300B herbal grinder from Dong Nam Co. Ltd (Vietnam). The powdered fruit was stored in moisture-resistant plastic bags at room temperature.

Preparation of the extract

The extraction of samples from 250 g of *Caryota urens* fruit powder by immersing it in 1000 mL of 96% ethanol, sealed and stored for one week, concurrently subject to continuous agitation. The filtration process involved a series of cotton filters and subsequent Whatman No.1 filter paper, with the filtered solution evaporated using a rotary evaporator RE301B-T at 50°C (Yamato, Japan). The ethanol extract from *C. urens* fruits (designated as ECUF) was then preserved in a moisture-resistant container and stored at 4°C until utilized for subsequent experiments.

Phytochemical screening and quantitative analysis in the ethanol extract from C. urens fruits

Phytochemical screening: The ethanol extract from *C. urens* fruits (ECUF) underwent qualitative phytochemical analysis, following established procedures as described in the study by Nhung and Quoc.[10] This analysis aimed to determine the presence of various diverse chemical components, including alkaloids, flavonoids, tannins, terpenoids, saponins, cardiac glycosides, steroids, and phenolics in the extract.

To assess the presence of alkaloids, 5 mL of the extract solution was dissolved in 3 mL of acidified ethanol, then gently heated and filtered. Several drops of Mayer's reagent and 1 mL of Dragendroff's reagent were added to 1 mL of the filtered solution, and the appearance of turbidity was observed. Flavonoid analysis involved adding a few drops of 1% aluminum chloride solution to 5 mL of the extract solution. The appearance of a yellow color indicated the presence of flavonoids. For tannin testing, a few drops of 0.1% iron chloride were added to the extract solution, and the development of a brown or blackish color was observed, indicating the presence of tannins. Saponin testing included boiling 2 g of the sample in 20 mL of distilled water, followed by filtration. Subsequently, 10 mL of the filtrate was mixed with 5 mL of distilled water and vigorously shaken to produce stable foam. This frothy portion was then combined with 3 drops of olive oil and shaken vigorously again to observe the formation of an emulsion. To test for the presence of steroids, 2 mL of acetic anhydride was added to 0.5 g of ethanol extract solution of each sample with 2M H_2SO_4. The color change from purple to blue or green in some samples indicates the presence of steroids. The method for testing terpenoids involves mixing 5 mL of the extract solution with 2 mL of chloroform and then carefully adding concentrated H_2SO_4 (3 mL) to form a layer. The formation of a reddish-brown color on the surface indicates a positive result for the presence of terpenoids. In the cardiac glycoside test, 5 mL of the plant sample was mixed with 2 mL of acetic acid containing a drop of ferric chloride solution. Then, the solution was reinforced with 1 mL of concentrated sulfuric acid. A brown ring appeared at the interface, indicating the presence of deoxysugar, a characteristic of cardenolides. A potential purple ring may appear below the brown ring and in the acid layer, and the gradual development of a greenish-blue ring throughout the thin layer can be observed. The phenolic test involves mixing 1 mL of the extract solution with 2 mL of 10% lead acetate solution and observing a brown precipitate, indicating a positive reaction.

Phytochemical quantification: To quantify the total phenolic content, we adhered to the Folin-Ciocalteu colorimetric method as described in the study by Nhung and Quoc.[11] Specifically, 0.3 mL of the extract solution was mixed with 2.25 mL of Folin-Ciocalteu phenol reagent. After incubating for 5 minutes, 2.25 mL of 6% sodium carbonate was added to the mixture and left at room temperature for 90 minutes. The absorbance of the solution was then measured at 725 nm. A standard curve for gallic acid was also established within the concentration range of 0-200 µg/mL using a similar procedure. The analysis results were reported in milligrams of gallic acid equivalent (GAE) per gram of extract.

To assess the total flavonoid content, we employed the aluminum colorimetric method, which was modified based on the original method by Nhung and Quoc, with specific adjustments.[11] Quercetin was used as the standard reference, and a standard curve for quercetin was constructed within the concentration range of 0-200 µg/mL. For this method, 0.5 mL of the extract solution and 0.5 mL of the standard were separately placed into individual test tubes. Then, to each tube, 10% aluminum chloride (0.1 mL), 1 M potassium acetate (0.1 mL), 80% methanol (1.5 mL), and distilled water (2.8 mL) were added and thoroughly mixed. A blank sample was prepared similarly, substituting 0.5 mL of distilled water for the sample or standard, and replacing aluminum chloride with distilled water. All test tubes were then incubated at room temperature for 30 minutes, followed by measuring the absorbance at 415 nm. The flavonoid concentration was reported as milligrams of quercetin equivalent (QE) per gram of extract.

The assessment of terpenoid content began by combining 200 µL of the ethanol extract solution (0.1 mg/mL) with 1 mL of perchloric acid and 300 µL of a vanillin/acetic acid solution (5% w/v). Following this, 5 mL of glacial acetic acid was added, and the absorbance was measured at 548 nm using a Shimadzu UV-visible spectrophotometer (Shimadzu Corporation, Japan). The quantification of terpenoids relied on a standard curve derived from varying concentrations (ranging from 0.0625 to 1 mg/mL) of ursolic acid.[12]

Experimental animals

In this study, we utilized Swiss albino mice weighing between 30 and 32 g. The animals were sourced from the Pasteur Institute, Ho Chi Minh

City (Vietnam) (Coordinates: 10°47'10.3"N 106°41'19.3"E), and were housed in wire-topped glass cages under standard environmental conditions (temperature of 25 ± 2ºC, relative humidity of 55 ± 5%, and a 12h/12h light-dark cycle). They were provided with free access to standard laboratory food obtained from the Pasteur Institute, Ho Chi Minh City, and water ad libitum, and were allowed to acclimatize to the laboratory environment for 7 days. All experimental procedures complied with the guidelines and regulations set forth by the Institutional Animal Ethics Committee at Ho Chi Minh City University of Industry, Vietnam. Additionally, animal experiments were conducted following ethical principles in animal research, as outlined in the Basel Declaration on Animal Research by Allison.[13]

Induction of arthritis and experimental design

To induce arthritis using CFA, a single dose of 1 mL CFA (Sigma Aldrich, St. Louis, MO) was injected subcutaneously into the right hind paw. Injection of CFA at this site for approximately 7 days resulted in a pathology resembling low-grade arthritis in the hind paws of the mice, manifested by persistent edema and increased stiffness throughout the experiment. The severity of edema in the paws was measured using digital calipers.[14]

After inducing arthritis for 7 days, the experimental animals were divided into 6 groups (each group consisting of 5 mice), including 1/ Normal group: that received no treatment or intervention. 2/ Arthritis control group (RA group): mice were subcutaneously injected with CFA (0.1 mL, single dose) into the right hind paw to induce arthritis. This group was also provided with physiological saline (10 mL/kg body weight) via oral administration daily. 3/ Arthritis group treated with Mobic (RA+Mobic group): mice were injected with CFA (0.1 mg)/kg) and treated with Mobic (0.2 mg/kg) orally daily.[15] 4-6/ Arthritis groups treated with ethanol extract of *C. urens* fruit (ECUF) (RA+ECUF 100-300 groups): mice were injected with CFA (0.1 mg)/kg and treated with ECUF (100, 200, and 300 mg/kg, respectively) orally daily. The animals were continuously treated daily until day 28 of the experiment. The degree of swelling and joint diameter were regularly recorded.

Measurement of body weight

The body weight of mice was measured once every 7 days using the Sartorius Entris 3202i-1S electronic scale (Sartorius group, Germany). The percentage of body weight gain (WG) was calculated using the following formula (1):[16]

$$\text{WG}\ (\%) = \frac{\text{Body weight of each week} - \text{Initial body weight (g)}}{\text{Initial body weight (g)}} \times 100 \quad (1)$$

Measurement of body and hind paw temperature

A portable digital thermometer (ATK-610B, ATP Instruments, USA) was used for measuring temperature. Body temperature was checked with a rectal probe. Temperature at the lateral side of the foot was measured with thermocouple probes.[16]

Homogenization of tissue

The joint, liver, kidney, and spleen tissue of mice were collected following euthanasia by CO_2 inhalation. Immediately afterward, the joints, livers, kidneys, and spleens were dissected and placed in 0.9% sodium chloride solution pre-chilled to ice temperature. The tissue was stored at -80°C until further processing. Subsequently, the tissue was homogenized in 50 mM phosphate buffer at pH 7.0, supplemented with 0.1 mM EDTA, to obtain a 5% homogenate solution for malondialdehyde (MDA) assays. Superoxide dismutase (SOD) activity was assessed in the supernatant obtained after centrifugation of the 5% homogenate at 600 × g for 10 minutes at 4°C. The supernatant was then collected and used for subsequent analysis.[17]

Lipid peroxidation (LPO)

Malondialdehyde (MDA) was determined using the method described in the study by Kesherwani *et al*., with propan 1,1,3,3-tetramethoxy as the standard.[4] In summary, 8.1% SDS (sodium dodecyl sulfate) was added to the homogenization tissue sample and incubated for 10 minutes at room temperature (RT), followed by boiling with 20% acetic acid and 0.6% thiobarbituric acid for 60 minutes in a water bath (Thermo Fisher Scientific, USA). After cooling, butanol: pyridine (15:1 v/v) was added, and the sample was centrifuged at 600 × g for 5 minutes. The absorbance of the upper colored layer was measured at 532 nm, and the MDA concentration was expressed as nM/mg protein.

Antioxidants estimation

*Total glutathione (GSH)***:** The concentration of glutathione (GSH) was quantified using a recycling assay with dithionitrobenzoic acid, following the method described by Yonar *et al*. with minor modifications.[18] In this procedure, the absorbance at 412 nm wavelength of the colored product resulting from the reaction between the DTNB reagent and the sulfhydryl group of GSH was measured. One milliliter of the sample was deproteinized by adding a solution containing 1.67 g metaphosphoric acid, 0.2 g Na_2EDTA, and 30 g NaCl in distilled water. Na_2HPO_4 (2.4 mL) and 0.3 mL DTNB were added to the upper layer, followed by centrifugation (10 minutes, 3000 g/minute). The formation of 5-thio-2-nitrobenzoic acid, proportional to the GSH concentration, was monitored at 412 nm wavelength, at 25°C, with a blank sample as a control for the reagent.

Total protein estimation (TP): The total protein concentration was estimated using the method described by Middha *et al*. with slight modifications, employing bovine serum albumin (BSA) as the standard.[17] In summary, 1 mL of the sample supernatant was added to 5 mL of alkaline sulfate solution and incubated for 10 minutes at room temperature. Subsequently, 0.6 mL of diluted Folin-Ciocalteu reagent (1:1 ratio) was added and incubated for 30 minutes at room temperature. The absorbance of the solution was measured at 660 nm against a blank sample.

Antioxidant enzymes activity

Glutathione peroxidase (GPx): The activity of glutathione peroxidase (GPx) was measured at 37°C using the method described by Middha *et al*. with minor adjustments.[17] The reaction mixture consisted of 500 µL phosphate buffer solution, 100 µL of 0.01 M reduced glutathione (GSH), 100 µL of 1.5 mM NADPH, and 100 µL of glutathione reductase (GR) (0.24 U). Then, 100 µL of tissue extract solution was added to the reaction mixture and incubated at 37°C for 10 minutes. Fifty microliters of 12 mM t-butyl hydroperoxide were added to 450 µL of the tissue reaction mixture and measured at 340 nm wavelength for 180 seconds using a spectrophotometer BioMate 160 (Themo, USA). The molar absorption coefficient of 6.22 × 103 M/cm was used to determine the enzyme activity. One unit of activity was defined as the amount of NADPH oxidized per minute per milligram of protein.

Total superoxide dismutase (SOD): The activity of superoxide dismutase (SOD) was measured using the method described by Middha *et al*. with slight modifications.[17] A buffer solution of 0.05 M carbonate (pH 10.2), supplemented with 0.1 mmol EDTA and 30 mmol epinephrine in 0.05% acetic acid, was added to the tissue extract solution, and the changes in SOD activity were measured at 480 nm wavelength for 4 minutes. This activity is represented by the amount of enzyme required to inhibit 50% of the epinephrine oxidation process, equivalent to one unit, and reported as units per milligram of protein.

Total catalase (CAT): The catalase (CAT) activity was assessed following the method outlined by Yonar *et al*.[18] The assay is based on determining the rate constant of hydrogen peroxide decomposition by catalase enzyme (CAT). In the experiment, 2 mL of the sample was added to 1 mL of 40 mM H_2O_2 in a phosphate buffer solution (50 mM, pH 7.0, adjusted by combining 0.681 g KH_2PO_4 in 100 mL and 1.335 g $Na_2HPO_4.2H_2O$ in 150 mL). The reduction of H_2O_2 was quantified through spectrophotometric analysis at 240 nm wavelength over 3 minutes.

Statistical analysis

Statistical analysis was conducted using Statgraphics Centurion XIX software. The results of each experiment were repeated five times and presented as mean ± standard deviation (SD). Data were analyzed for significance using the t-test or analysis of variance (ANOVA). Results with a p-value ≤ 0.05 were considered statistically significant.

Results and Discussion

Phytochemical screening and quantitative analysis in the ethanol extract from C. urens fruits

This study analyzed the ethanol extract from *C. urens* fruits (ECUF) and identified the presence of various important plant components. Results

revealed that ECUF contains compounds such as alkaloids, tannins, saponins, polyphenols, steroids, terpenoids, and flavonoids, while cardiac glycosides were not detected in the extract, as illustrated in Table 1. Furthermore, the contents of flavonoids, polyphenols, and terpenoids in ECUF were determined, as described in Table 2. The results showed that the concentrations of flavonoids were 42.82 ± 2.66 mf QE/g, polyphenols were 68.89 ± 3.74 mg GAE/g, and terpenoids were 70.36 ± 4.45 mg TAE/g. These findings provide important information regarding the chemical composition of ECUF, contributing to a better understanding of the biological and pharmacological potential of this plant species.

For millennia, the use of plants for treating various illnesses has been an integral part of humanity's healthcare history. This knowledge has been passed down through hundreds of generations and remains a primary method for healthcare for over 7 billion people worldwide today. Phytochemicals play a crucial role in imparting the unique characteristics of plants, including aroma, flavor, and biological activity. In recent years, there has been a clear trend in utilizing plants to develop new medicines in many countries worldwide. Some phytochemicals can regulate both inflammation and oxidative stress simultaneously, as these two phenomena are often interconnected and mutually reinforcing. For instance, reactive oxygen species (ROS) can act as signaling molecules for the inflammatory process, and thus, inflammation can induce oxidative stress and diminish the cell's antioxidant capacity.[19] Compounds derived from plants, such as alkaloids, phenols, flavonoids, saponins, tannins, terpenoids, and steroids, are renowned for their ability to prevent and treat various diseases. For instance, saponins participate in the development and defense mechanisms of plants, where they are synthesized to counteract attacks from bacteria, insects, or herbivores. They can reduce the concentration of pro-inflammatory cytokines and act as ligands for glucocorticoid receptors. Terpenoids stand out for their anti-inflammatory and antioxidant effects. Phenolic compounds contribute to protective mechanisms against bacterial attacks and stressful conditions by scavenging free radicals and chelating metals, thereby safeguarding plants from oxidative molecules. Additionally, they can modulate inflammatory pathways, such as NF-kB, and activate antioxidant pathways, such as Nrf-2.[20] Within this group, flavonoids and polyphenols have been recognized for their potent antioxidant activity, aiding in cellular protection against the impact of free radicals and enhancing overall health. Terpenoids also play a crucial role in cellular defense against oxidative stress by bolstering the body's antioxidant defense mechanisms. [21] In the context of joint inflammation, oxidative stress typically escalates due to the excessive generation of free radicals during the inflammatory and immune responses. The presence of secondary metabolites in the extract, such as flavonoids, polyphenols, and terpenoids, may alleviate oxidative stress and inflammation, thereby improving overall health and reducing symptoms of joint inflammation.

Body weight

The body weight and weight gain of the control animals with induced arthritis (CFA group) decreased steadily from day 7 until the final day of the study, as evidenced by the observations depicted in Figures 1A and 1B compared to the normal control group ($p < 0.05$). Administration of the ethanol extract from *C. urens* fruit significantly preserved the body weight of the treated animals when compared to the arthritic control group ($p < 0.05$). The results indicate that the ethanol extract from *C. urens* fruit effectively mitigates the decline in body weight associated with arthritis induction. This suggests a potential therapeutic effect of the extract in managing arthritis-related weight loss, which is often observed in experimental models.

The relationship between body weight and weight gain is pivotal in the context of arthritis. In many cases of arthritis, animals often experience weight loss and diminished weight gain, indicating a decline in overall health and the detrimental impact of arthritis on the body. Weight loss reflects muscle loss caused by arthritis, thereby reducing muscle strength and mobility. Additionally, weight loss can result from reduced food intake due to pain and discomfort during movement. An unstable weight and decreased weight gain will adversely affect the quality of life. Complete Freund's Adjuvant (CFA) is a potent immunostimulant used to induce arthritis in research models. CFA elicits a robust immune response in animals, leading to the development of arthritis. CFA can impact body weight and weight gain through several mechanisms. Arthritis induced by CFA causes pain and discomfort in animals, resulting in reduced food intake and physical activity, leading to weight loss and diminished weight gain. Additionally, CFA stimulates the production of inflammatory mediators such as cytokines and prostaglandins, which promote inflammation and muscle loss, further contributing to decreased body weight. Arthritis also leads to muscle dysfunction, reducing muscle strength and mobility, ultimately resulting in weight loss and muscle wasting.[22] Therefore, maintaining or restoring body weight and weight gain a crucial goal in treating arthritis, enhancing the quality of life and activity levels in animals. Ethanol extract from *C. urens* fruit (ECUF) aids in increasing body weight through several mechanisms, including improving food consumption and physical activity, enhancing nutrient absorption, influencing energy balance, and exerting anxiolytic effects.

The phytochemicals present in ECUF possess anti-inflammatory properties, alleviating pain and discomfort in experimental animals, thereby enhancing food intake and physical activity. ECUF also enhances the absorption and utilization of nutrients from food, leading to improved growth and development in experimental animals.

Additionally, ECUF positively impacts energy metabolism and utilization in the body, resulting in weight gain through energy balance. Furthermore, ECUF exhibits anxiolytic effects or reduces stress, promoting a sense of comfort in experimental animals and consequently increasing body weight.

The body and hind paw temperature

Elevated body temperature is a characteristic indicator of inflammation, including rheumatoid arthritis in mice. Figures 2A and 2B illustrate the evaluation of overall body temperature and hind paw temperature in mice administered with normal saline, Mobic, and ethanol extract from *C. urens* fruit (ECUF). Mice with CFA-induced arthritis demonstrate significantly higher body temperature and hind paw temperature compared to those receiving normal saline ($p < 0.05$).

Table 1: Analysis of phytochemicals in ethanol extract solution from *C. urens* fruit through qualitative screening

Phytochemicals	Present in ECUF	Phytochemicals	Present in ECUF
Alkaloids	+	Cardiac glycosides	-
Tannins	+	Steroids	+
Saponins	+	Terpenoids	+
Polyphenols	+	Flavonoids	+

Note: Presence of phytochemicals in ECUF: (+) present and (-) absent

Table 2: Determining the concentrations of flavonoids, polyphenol, and terpenoids in the ethanol extract solution from *C. urens* fruit

Sample	Total flavonoid content (mg QE/g)	Total terpenoid content (mg TAE/g)	Total polyphenol content (mg GAE/g)
ECUF	42.82 ± 2.66	70.36 ± 4.45	68.89 ± 3.74

Note: GAE: Gallic acid equivalents, QE: Quercetin equivalents. TAE: Tannic acid equivalents.

Moreover, subsequent administration of ECUF results in a significant reduction in body temperature and hind paw temperature compared to the CFA-induced arthritis group ($p < 0.05$). The study highlights a substantial decrease in body temperature and hind paw temperature following ECUF administration compared to mice with CFA-induced arthritis. This suggests potential anti-inflammatory properties of ECUF, as indicated by its capability to alleviate the elevated body temperature and hind paw temperature associated with arthritis. These findings hold significance as they lend additional support to the prospective therapeutic application of ECUF in inflammation management and symptom alleviation, thereby fostering the development of innovative arthritis treatments.

Inflammation, a hallmark of arthritis, often triggers fever, resulting in heightened body temperature. Fever, a physiological response to inflammation, underscores the active engagement of the body's immune system in combatting the inflammatory process. Body temperature and hind paw temperature serve as critical indicators of inflammation in arthritis. Elevated body temperature signifies systemic inflammation, while increased hind paw temperature indicates localized inflammation in the joints. The hind paw temperature serves as a gauge for this localized inflammation, reflecting heightened blood flow to the affected joint, a characteristic trait of arthritis. Monitoring body temperature and hind paw temperature offers valuable insights into the severity and progression of joint inflammation, aiding in the assessment and management of arthritis.[23] The Complete Freund's Adjuvant (CFA) increases body temperature and hind paw temperature is primarily through its induction of inflammation. CFA contains heat-killed mycobacteria and other immunostimulatory components that provoke a robust immune response when injected into animals. Upon injection, CFA triggers the release of pro-inflammatory cytokines and chemokines, such as interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-α), and prostaglandins, among others. These molecules initiate and amplify the inflammatory process, leading to vasodilation, increased vascular permeability, and recruitment of immune cells to the site of injection, including the joints. In the joints, this inflammatory cascade results in the production of inflammatory mediators and the activation of resident immune cells, such as macrophages and synoviocytes, leads to swelling, redness, and increased blood flow in the affected joints, contributing to elevated hind paw temperature. Furthermore, the systemic release of pro-inflammatory cytokines lead to fever, causing an increase in body temperature. Fever is a common response to inflammation and serves to enhance the immune response by promoting the activity of immune cells and inhibiting the growth of pathogens.[16] The reduction of body temperature and hind paw temperature by the ethanol extract from *C. urens* fruit (ECUF) attributed to its multifaceted effects, including anti-inflammatory, antioxidant, cytokine-modulating, and vasodilatory properties. ECUF exhibits anti-inflammatory attributes, mitigating inflammation-induced fever and localized temperature elevation in the hind paws. By inhibiting pro-inflammatory mediators or pathways, ECUF attenuate the inflammatory response, resulting in decreased body temperature and hind paw temperature. Additionally, ECUF exerts antioxidant effects by scavenging reactive oxygen species (ROS) and reducing oxidative stress, which are implicated in inflammation and tissue damage, thereby indirectly lowering body temperature and hind paw temperature. ECUF regulates the production and release of cytokines involved in arthritis-related inflammation, such as interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-α), potentially reducing fever and localized inflammation. Furthermore, ECUF enhances blood flow and microcirculation in the joints, improving tissue perfusion and reducing localized temperature, thereby aiding in heat dissipation and inflammation reduction in the hind paws.

Lipid peroxidation (LPO)

These results suggest that injecting CFA beneath the liver induces significant inflammation, as evidenced by peak swelling observed on the 7th day. As illustrated in Figure 3, the concentration of MDA in joint tissues, liver, kidneys, and spleen of the negative control animals (RA group) significantly increased compared to the normal control group ($p < 0.05$), indicating elevated oxidative stress levels. However, treatment with ECUF effectively reduced MDA concentration compared to the diseased control mice (RA group) ($p < 0.05$), with the most significant reduction observed at a dose of 300 mg/kg ($p < 0.05$). This suggests that ECUF has antioxidant properties, as it was able to mitigate oxidative stress induced by arthritis. The optimal dose of ECUF, at 300 mg/kg, demonstrated comparable efficacy to the standard drug Mobic (2.0 mg/kg) ($p > 0.05$) in reducing MDA concentration, highlighting its potential as a therapeutic agent for arthritis.

Oxidative stress denotes an imbalance between antioxidants and pro-oxidants, fostering conditions favorable for the weakening of antioxidant defenses, thereby diminishing the activities of signal transduction molecules and enzymes, consequently leading to tissue damage. Reactive oxygen species (ROS) represent the primary molecular entities responsible for oxidative stress, generated under physiological and pathological conditions such as cellular metabolism. ROS originate intrinsically within the body including mitochondria, endoplasmic reticulum, and peroxisomes, where enzymatic reactions and auto-oxidation processes of various compounds occur.

Extrinsic factors such as UV exposure, stress, infections, allergies, and pollution also contribute to ROS generation. ROS plays a dual role in intracellular milieu equilibrium, executing both beneficial and detrimental effects. They participate in cellular physiological responses to pathological stimuli, activating immune cells such as neutrophils, macrophages, and T lymphocytes. They stimulate cell proliferation and apoptosis, concurrently modulating numerous cell signaling pathways, particularly those associated with JNK (c-Jun N-terminal kinase) and p38 MAPK (Protein kinase c-Jun N-terminal kinase). Adverse effects of ROS include nucleic acid, protein, and lipid damage. When oxidative damage surpasses repair capacity, cellular injury occurs. The imbalance between oxidation and reduction is implicated in various diseases, aging processes, and cancer development. Additionally, excessive or chronic ROS production is a primary mechanism underlying the progression of inflammatory diseases.[24]

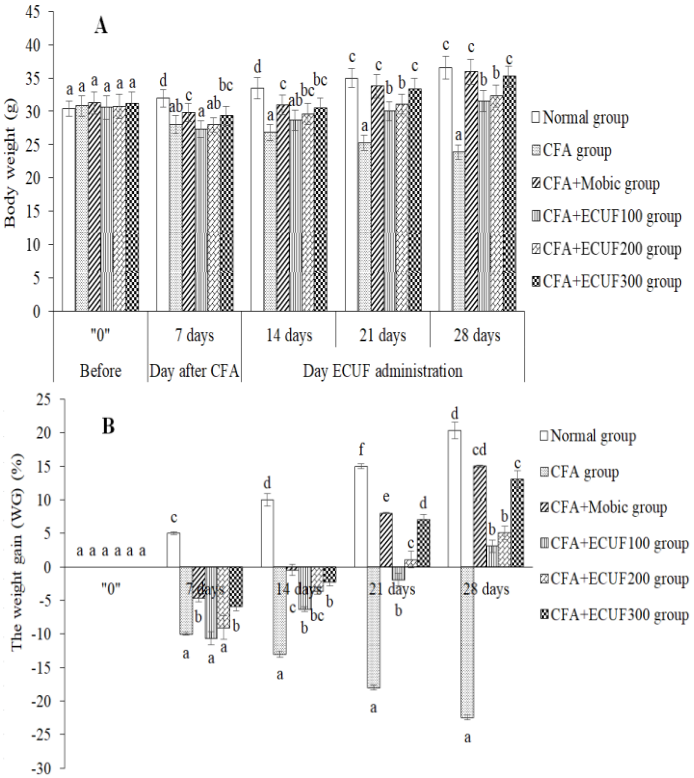

Figure 1: Ethanol extract from *C. urens* fruit impacts on body weight (A) and body weight gain (B) in CFA-induced arthritis mouse model. Results are expressed as Mean ± SD, with letters (a, b, c, d, e, and f) indicating statistically significant differences among treatments ($p < 0.05$).

Within biological processes, lipids, particularly polyunsaturated fatty acids with multiple carbon-carbon double bonds, play pivotal roles and are profoundly affected by oxidative stress. Oxidizing agents induce the formation of unstable lipid radicals (L-) by abstracting hydrogen atoms, subsequently generating lipid peroxyl radicals (LOO-) upon incorporation of an oxygen molecule. These radicals further react by scavenging hydrogen atoms from other lipid molecules, yielding more stable compounds known as lipid hydroperoxides (LOOHs), a process termed lipid peroxidation. Both lipid hydroperoxides and peroxyl radicals can undergo cyclization and fragmentation processes, giving rise to secondary products. Malondialdehyde (MDA) is a compound arising from lipid peroxidation processes, possessing mutagenic and cytotoxic properties. Upon formation, MDA can undergo biotransformation through various enzymes such as aldehyde dehydrogenase, or engage in chemical interactions with proteins and nucleic acids. This leads to the formation of cross-links between DNA and proteins, as well as the generation of other addictive products, all of which inflict damage upon biological molecules.[25] The heightened levels of MDA in organs such as joints, liver, kidneys, and spleen of mice in arthritic conditions reflect an escalation in oxidative stress and corresponding tissue damage in these organs. MDA, as a hallmark product of lipid oxidation, typically surges when the oxidative balance in the body is disrupted. Thus, the increased concentration of MDA in the joints of arthritic mice may signify an augmentation of oxidative stress and inflammation, along with tissue injury within the joints. Manifestations such as swelling, pain, and impaired joint function, especially evident in inflammatory joint diseases like rheumatoid arthritis and polyarticular juvenile idiopathic arthritis, may accompany this. Furthermore, the heightened MDA levels in the liver, kidneys, and spleen also indicate that these organs are facing oxidative stress and oxidative damage during joint inflammation. The elevation of MDA in arthritic joints induced by the pro-inflammatory agent, CFA delineates a state of natural oxidative stress in the body, reflecting inflammation and heightened cellular damage. This mechanism represents a natural response of the body to cope with oxidative stress. Excessive production of MDA may contribute to health issues such as inflammation and cellular damage commonly observed in arthritic conditions.[26] The mechanism of action of ethanol extract from *C. urens* fruit (ECUF) is associated with the reduction of malondialdehyde (MDA) concentration in the bodies of mice with arthritis. ECUF contains bioactive compounds with antioxidant properties, combating oxidative stress and lipid peroxidation in the body. By scavenging free radicals and inhibiting the lipid peroxidation process, ECUF aids in reducing the production of MDA. This reduction in MDA levels signifies the minimization of cellular damage caused by oxidative stress and inflammation in arthritic conditions. The antioxidant activity of ECUF plays a crucial role in alleviating oxidative stress and protecting overall joint health in arthritic mice.

Antioxidants estimation

Table 3 illustrates a significant reduction ($p < 0.05$) in the concentrations of total glutathione (GSH) and total protein (TP) in joint, liver, kidney, and spleen tissues observed in the diseased control mice (RA group) compared to the vehicle control mice (normal group). The modulatory effect on GSH and TP concentrations increased in the treatment groups receiving ECUF in a dose-dependent manner ($p < 0.05$). Maximum efficacy was noted with the 300 mg/kg extract dose, equivalent to the standard drug Mobic ($p < 0.05$). These findings hold significance as they suggest that ethanol extract from *C. urens* fruit (ECUF) has the potential to restore the levels of total glutathione (GSH) and total protein (TP) in tissues affected by arthritis. This restoration of GSH and TP levels indicates a potential protective effect of ECUF against oxidative stress and tissue damage associated with arthritis.

Total glutathione (GSH) and total protein (TP) serve as fundamental biomarkers frequently examined within the realm of oxidative stress research. Comprised of glutamate, cysteine, and glycine, glutathione (GSH) assumes a pivotal role in the body's antioxidant defense mechanism. Functioning as a robust antioxidant, GSH neutralizes free radicals and reactive oxygen species (ROS), safeguarding cellular structures against oxidative harm. Moreover, GSH contributes to detoxification pathways by binding to harmful compounds and facilitating their excretion from the organism. The quantification of total glutathione levels indicates the overall antioxidant capacity present within cells and tissues.

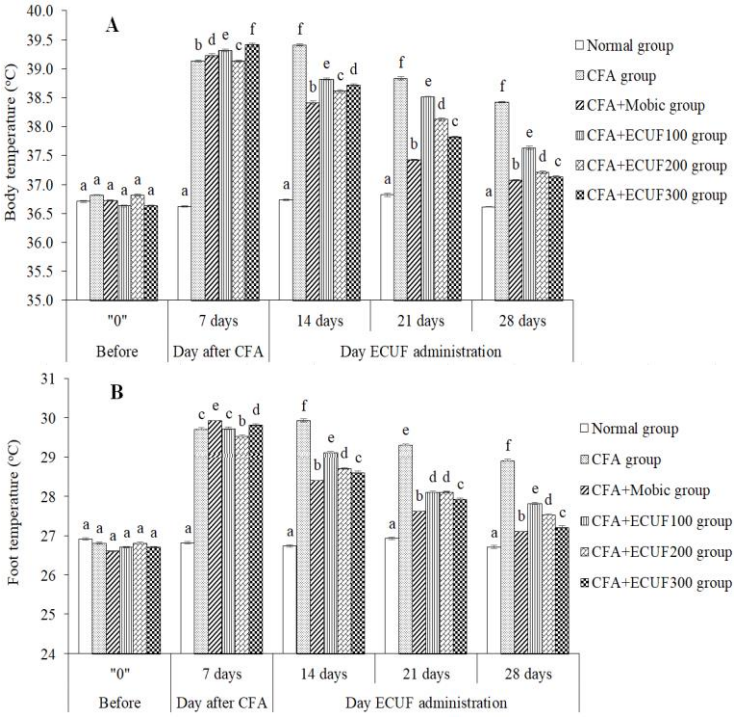

Figure 2: Influence of ethanol extract from *C. urens* fruit on body temperature (A) and hind paw temperature (B) in CFA-induced arthritis mouse model. Results are expressed as Mean ± SD, with letters (a, b, c, d, e, and f) indicating statistically significant differences among treatments ($p < 0.05$).

Table 3: The influence of ethanol extract from *C. urens* fruit on antioxidant compounds in the joint, liver, kidney, and spleen tissues of CFA-induced arthritic mice

Treatments	Total glutathione (GSH) (nM/mg tissue)				Total protein (TP) (mg/mL)			
	Joints	Livers	Kidneys	Spleens	Joints	Livers	Kidneys	Spleens
Normal group	8.38 ± 1.58^{c}	5.09 ± 1.08^{e}	7.09 ± 1.25^{d}	6.64 ± 1.14^{e}	17.28 ± 4.45^{c}	9.79 ± 2.64^{e}	14.63 ± 4.15^{c}	13.69 ± 3.76^{d}
RA group	2.03 ± 0.12^{a}	1.23 ± 0.05^{a}	1.72 ± 0.07^{a}	1.61 ± 0.08^{a}	4.19 ± 0.22^{a}	2.55 ± 0.12^{a}	3.54 ± 0.15^{a}	3.31 ± 0.17^{a}
RA+Mobic group	7.75 ± 1.54^{c}	4.45 ± 0.29^{de}	6.42 ± 0.77^{cd}	5.24 ± 0.54^{d}	15.42 ± 2.24^{c}	7.67 ± 1.25^{d}	12.42 ± 2.87^{c}	12.67 ± 1.62^{d}
RA+ECUF100 group	3.84 ± 0.44^{c}	2.33 ± 0.22^{b}	3.25 ± 0.32^{b}	3.04 ± 0.33^{b}	7.91 ± 1.57^{b}	4.83 ± 0.64^{b}	6.69 ± 1.48^{ab}	6.27 ± 1.35^{b}
RA+ECUF200 group	4.87 ± 0.52^{b}	3.26 ± 0.25^{c}	4.12 ± 0.64^{b}	3.86 ± 0.33^{c}	10.04 ± 3.45^{b}	5.74 ± 0.83^{bc}	8.51 ± 2.35^{b}	8.96 ± 2.28^{bc}
RA+ECUF300 group	7.341 ± 1.44^{c}	4.16 ± 0.34^{d}	5.86 ± 0.54^{c}	4.97 ± 0.42^{d}	14.34 ± 2.15^{c}	7.14 ± 1.25^{cd}	11.95 ± 2.72^{c}	11.46 ± 1.55^{cd}

The values are expressed as Mean ± SD, where the letters a, b, c, d, and e indicate differences between treatments ($p < 0.05$).

Total protein (TP) denotes the aggregate quantity of protein within a given biological specimen. Proteins represent indispensable macromolecules engaged in diverse cellular functions, encompassing enzymatic reactions, signal transduction, structural reinforcement, and immunological responses. Amidst oxidative stress scenarios, fluctuations in TP levels may signify shifts in cellular metabolic activity, protein biosynthesis, or proteolytic degradation pathways. The assessment of TP concentrations offers valuable insights into the holistic wellness and resilience of cells amidst oxidative stress challenges. These biomarkers serve as crucial indicators of oxidative injury, furnishing vital insights into the pathophysiology of oxidative stress-related ailments such as arthritis. The diminished concentrations of GSH and TP observed in the joint, liver, kidney, and spleen tissues of CFA-induced arthritic mice signify noteworthy declines, suggestive of adverse impacts on both antioxidant mechanisms and protein synthesis pathways within these tissues.

This implies the onset of oxidative stress alongside attenuated antioxidant defenses, culminating in tissue impairment and functional disruption amidst arthritic states.[27] The ethanol extract from *C. urens* fruit (ECUF) restores glutathione (GSH) and total protein (TP) levels in the joint, liver, kidney, and spleen tissues of arthritic mice, attributed to its antioxidative properties and regulatory potential on cellular metabolism. ECUF contains bioactive compounds with potent antioxidative properties capable of scavenging reactive oxygen species (ROS) and reducing oxidative stress levels in inflamed tissues. By neutralizing ROS, ECUF mitigates oxidative damage to cells and tissues, thereby preventing the decrease in GSH and TP levels. Furthermore, ECUF may modulate cellular metabolism and protein synthesis processes, leading to the restoration of TP concentrations in inflamed joint tissues, either by stimulating protein synthesis or inhibiting protein degradation pathways, contributing to TP maintenance. Additionally, ECUF may directly enhance the synthesis or availability of GSH, a critical antioxidant molecule within cells, promoting GSH production or preventing its decline. This ensures the availability of this antioxidant compound to neutralize ROS and protect cells from oxidative damage. Thus, ECUF's ability to restore GSH and TP levels in joint tissues is associated with its antioxidative effects, modulation of cellular metabolism, and regulation of protein synthesis pathways, collectively preserving tissue integrity and function under oxidative stress conditions associated with arthritis.

Antioxidant enzymes activity

The impact of oxidative stress and the reduction in antioxidant enzyme levels influenced by the action of ethanol extract from *C. urens* fruit (ECUF) is depicted in Table 4 and Figure 4. Oxidative stress contributes to a reduction in the activity of antioxidant enzymes, as evidenced by decreased levels of glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) in the diseased control mice compared to the vehicle control group ($p < 0.05$). However, treatment with both Mobic and different doses of ECUF successfully restored the activity of these antioxidant enzymes ($p < 0.05$). Particularly, the highest dose of ECUF (300 mg/kg) showed comparable efficacy to the standard drug Mobic ($p > 0.05$) in restoring the antioxidant enzyme levels. This suggests that ECUF possesses potent antioxidant properties, which could be beneficial in mitigating oxidative stress-related disorders such as arthritis.

Rheumatoid arthritis (RA) is an autoimmune condition characterized by the immune system's erroneous targeting of healthy tissues in the body, resulting in inflammation of the joint lining, bone erosion, and joint deformities. Substantial evidence underscores the pivotal role of oxidative stress and disruptions in antioxidant equilibrium in the disease's pathogenesis. Oxidative stress arises from an imbalance between the generation of free radicals and the body's antioxidant defense mechanisms. This imbalance stems from the excessive production of diverse reactive oxygen species (ROS), including superoxide anion ($O^{2\bullet-}$), hydroxyl radical ($OH^{\bullet}$), and hydrogen peroxide (H_2O_2). $O^{2\bullet-}$, a primary ROS, originates from various sources, while H_2O_2, although not a free radical per se, can produce $OH^{\bullet}$ through the Fenton reaction in the presence of Fe^{2+}. In each cell, enzymatic mechanisms are present to counteract excessive reactive oxygen species (ROS) and shield against oxidative stress-induced damage. Among these mechanisms, key protective enzymes include superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). SOD, a metalloprotein, functions to eliminate superoxide anion ($O2^{\bullet-}$) by converting it into hydrogen peroxide (H_2O_2) and oxygen molecules. Subsequently, H_2O_2 is further broken down into water by the combined actions of CAT and GPx.

Through the coordinated activity of these enzymes, not only are harmful ROS like $O^{2\bullet-}$ and H_2O_2 transformed into innocuous water, but also the generation of the highly reactive hydroxyl radical ($OH^{\bullet}$) is thwarted. Catalase (CAT) plays a crucial role in neutralizing elevated concentrations of both externally-derived and internally-produced hydrogen peroxide (H_2O_2), while glutathione peroxidase (GPx) contributes to the elimination of lower levels of internally-generated H_2O_2. Moreover, the activity of GPx is modulated by the levels of glutathione (GSH) and the functioning of glutathione reductase (GR).[28] The concentrations of GPx, SOD, and CAT in the joint, liver, kidney, and spleen tissues of mice with CFA-induced arthritis reflect a critical issue about the antioxidant defense mechanism. Specifically, the reduction in the levels of these antioxidant enzymes indicates a diminished capacity of the body to neutralize reactive oxygen species (ROS) and oxidative stress. This imbalance in the antioxidant defense mechanism may exacerbate tissue damage and inflammatory conditions associated with arthritis. Therefore, restoring the activity of GPx, SOD, and CAT could be highly significant in mitigating oxidative stress and preserving joint health in arthritis conditions.[29]

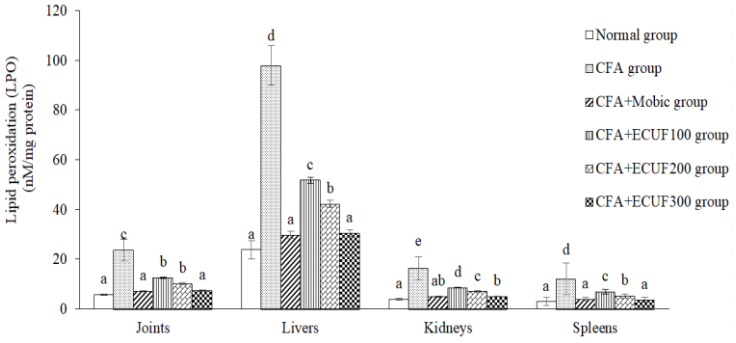

Figure 3: The impact of ethanol extract solution from *C. urens* fruit on the malondialdehyde (MDA) levels in the joint, liver, kidney, and spleen tissues of CFA-induced arthritic mice. Results are expressed as Mean ± SD, with letters (a, b, c, d, e, and f) indicating statistically significant differences among treatments ($p < 0.05$).

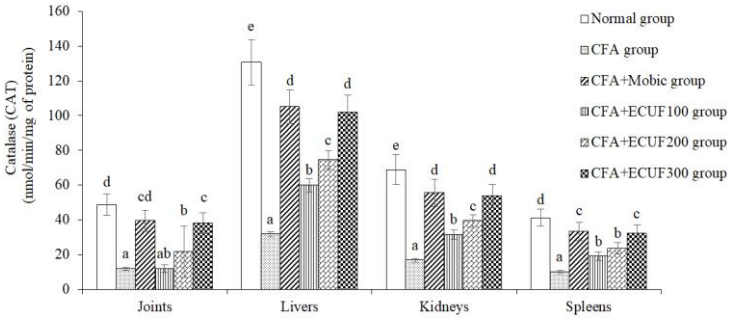

Figure 4: The effect of ethanol extract from *C. urens* fruit on the catalase (CAT) levels in the joint, liver, kidney, and spleen tissues of CFA-induced arthritic mice. Results are expressed as Mean ± SD, with letters (a, b, c, d, e, and f) indicating statistically significant differences among treatments ($p < 0.05$).

Table 4: The impact of ethanol extract from *C. urens* fruit on the levels of glutathione peroxidase (GPx) and superoxide dismutase (SOD) in the joint, liver, kidney, and spleen tissues of CFA-induced arthritic mice

Treatments	Toatal glutathione peroxidase (GPx) ((mM/mg protein)				Total superoxide dismutase (SOD) (U/mg protein)			
	Joints	Livers	Kidneys	Spleens	Joints	Livers	Kidneys	Spleens
Normal group	8.75 ± 0.72^{e}	5.31 ± 0.37^{e}	7.32 ± 0.55^{e}	6.89 ± 0.45^{e}	7.64 ± 0.65^{e}	2.07 ± 0.12^{e}	2.29 ± 0.13^{e}	8.28 ± 0.81^{e}
RA group	2.12 ± 0.28^{a}	1.29 ± 0.17^{a}	1.77 ± 0.19^{a}	1.67 ± 0.18^{a}	1.85 ± 0.25^{a}	0.51 ± 0.03^{a}	0.55 ± 0.05^{a}	2.01 ± 0.23^{a}
RA+Mobic group	7.05 ± 0.57^{d}	4.27 ± 0.37^{d}	5.89 ± 0.35^{d}	5.55 ± 0.47^{d}	6.15 ± 0.49^{d}	1.67 ± 0.07^{d}	1.85 ± 0.08^{d}	6.67 ± 0.38^{d}
RA+ECUF100 group	4.01 ± 0.18^{b}	2.43 ± 0.14^{b}	3.35 ± 0.27^{b}	3.15 ± 0.25^{b}	1.85 ± 0.15^{b}	0.95 ± 0.11^{b}	1.05 ± 0.11^{b}	3.79 ± 0.31^{b}
RA+ECUF200 group	4.99 ± 0.41^{c}	3.03 ± 0.27^{c}	4.18 ± 0.36^{c}	3.93 ± 0.33^{c}	4.36 ± 0.37^{c}	1.18 ± 0.07^{c}	1.31 ± 0.11^{c}	4.73 ± 0.37^{c}
RA+ECUF300 group	6.82 ± 0.58^{d}	4.14 ± 0.34^{d}	5.71 ± 0.49^{d}	5.37 ± 0.47^{d}	5.96 ± 0.52^{d}	1.61 ± 0.11^{b}	1.79 ± 0.07^{d}	6.46 ± 0.57^{d}

The values are expressed as Mean ± SD, where the letters (a, b, c, d, and e) indicate differences between treatments ($p < 0.05$).

The ethanol extract from *C. urens* fruit (ECUF) restores GPx, SOD, and CAT concentrations in arthritic mice's joints, liver, kidney, and spleen tissues. ECUF's antioxidant properties scavenge reactive oxygen species (ROS), reducing oxidative stress. This relieves the burden on antioxidant enzymes like GPx, SOD, and CAT, enhancing their effectiveness in neutralizing ROS and repairing oxidative damage. Additionally, ECUF may boost the synthesis or activation of antioxidant enzymes, further improving their activity. This contributes to the restoration of GPx, SOD, and CAT levels in affected tissues. Overall, ECUF's antioxidant effects are vital for reducing oxidative stress, preserving tissue health, and combating arthritis-induced oxidative stress. This highlights ECUF's potential therapeutic value in managing arthritis-associated oxidative stress.

Conclusion

In this experimental investigation, our results validate that pretreatment with ethanol extract from *C. urens* fruit (ECUF) significantly attenuated the production of CFA-induced malondialdehyde (MDA), consequently reducing cellular lipid peroxidation and preventing cellular damage. Furthermore, ECUF pretreatment elicited a notable decrease in body temperature and hind paw temperature, accompanied by an increase in body weight. Moreover, it enhanced the activity of antioxidant enzymes and molecules such as glutathione (GSH), total protein (TP), catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD) in joint, liver, kidney, and spleen tissues of arthritic mice. Overall, the outcomes of this research lay the groundwork for further exploration of ECUF as a promising therapeutic avenue for managing oxidative stress in arthritis. Future studies could delve deeper into the underlying molecular mechanisms of ECUF's antioxidant properties and its potential efficacy in clinical settings. Additionally, investigations into the safety profile, optimal dosage, and long-term effects of ECUF supplementation would be valuable for advancing its development as a therapeutic intervention for arthritis-related oxidative stress.

Conflict of Interest

The authors declare no conflict 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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