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Research Article

Exploring The Anti-Arthritic Potential Of *Merremia Emarginataburm. Fin* Extract: Insights Into Molecular Modulation Of Proinflammatory Cytokines And Acute Phase Proteins In Adjuvant-Induced Arthritis Rat Model

Purushoth Prabu T.*[1], Deepak Venkataraman N.[2], Selvakumari E.[3], RavichandranS.[4], Kabilan N.[5], Manikgantan E. M.[6]

[1]*Department of Pharmacognosy, C L Baid Metha College of Pharmacy, Chennai, Tamilnadu, India.*
[2]*Department of Pharmacology, GRT Pharmaceutical and Educational Research, Thiruthani, Tamilnadu, India*
[3]*Department of Pharmacognosy, Mother Theresa Postgraduate and Research Institute of Health sciences, Puducherry, India*
[4]*College of Pharmacy, St. Mary's College of Medical Science, Puliangudi, Tenkasi, Tamilnadu, India*
[56]*Department of Siddha, The Tamil Nadu Dr MGR Medical University, Chennai, India*

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ABSTRACT

Background: A protective response to tissue damage is inflammation. An inflammatory response is caused by a variety of factors, all of which could harm tissue and result in the associated symptoms. the pathogenic parallels between mammalian rheumatoid arthritis and Freund's adjuvant-induced inflammatory patterns.

Results: When Freund's adjuvant was used to induce rheumatoid arthritis, analysis of the Paw volume, hematopoietic parameters, acute phase proteins, immunoglobulins, inflammatory mediators, lysosomal enzymes, plasma protein bound carbohydrates, and urinary collagen degradative products showed a notable beneficial effect. Additionally, the radiographic and histological analyses showed that the bone, collagen, and cartilage profiles had been significantly restored. These results point to the tested drugs' positive effects on numerous elements of the disease and point to their intriguing potential for treating rheumatoid arthritis. **Conclusions**: *Merremia emarginata* (EME) extract and fractions, particularly EME (400mg/kg) and MFME (100mg/kg), showed a reduction in the release of lysosomal enzymes, which resulted in a decrease in the degradation of cartilage, bone, and synovial tissue. In the end, this led to increased collagen and GAG synthesis. The observed improvement in the antioxidant system and the inhibition of the pro-inflammatory cytokines TNF- and IL-1 also support Merremia emarginata's

***Corresponding Author:** Purushoth Prabu T
Address: *Department of Pharmacognosy, C L Baid Metha College of Pharmacy, Chennai, Tamilnadu, India.*
Email ✉: pharmpurushoth@gmail.com

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anti-arthritic activity. The powerful protective effects of this plant may be attributable to the synergistic interaction of polyphenols like flavonoids, tannins, and phenolic acids like chlorogenic acid and quercetin, as well as the presence of Vitamin C and metals like zinc and copper. Overall, these data indicate Merremia emarginata's potential for treating rheumatism and verify its traditional medicinal use.

INTRODUCTION

Background

As a biological defense mechanism to get rid of or control dangerous substances, inflammation is a local reaction of living mammalian tissues to injury. Edema formation, leukocyte infiltration, and granuloma formation are a few factors that contribute to the inflammatory response and can all result in tissue damage and related symptoms [1]. An autoimmune condition known as rheumatoid arthritis is characterized by cartilage degradation, synovial lining hyperproliferation, and chronic inflammation. Tumor necrosis factor (TNF), in particular, is raised in synovial fluid and is thought to contribute to the development of the disease by upregulating a number of inflammatory mediators [2,3]. In mammals, Freund's adjuvant causes an inflammatory response resembling arthritis [4]. Due to its financial advantages and negligible side effects, research into plant-based medications utilized in conventional treatment has gained momentum. The World Health Organization (WHO) estimates that 80% of the world's population uses herbal treatments to treat a variety of medical issues [5]. One similar plant is the perennial, heavily branching herb or creeper Merremia emarginata Burm. F (Convolvulaceae). It can be found all over India, but it is most common in the moist areas of the upper Gangetic plain, Gujarat, Bihar, West Bengal, Western Ghats, and regions that rise up to 900 meters in the hills of Goa, Karnataka, India, Ceylon, and Tropical Africa [6]. It's crucial to keep in mind though that Merremia emarginata is occasionally contaminated with Centella asiatica [7]. It has been said that this plant possesses a number of significant therapeutic characteristics that are used in traditional indigenous medical practices. Cough, headache, neuralgia, rheumatism, diuretic conditions, inflammations, nasal issues, liver enlargement-induced fever, and kidney illnesses are among the list of claimed benefits. varied plant parts, such the leaves and roots, have varied uses, such as leaf powder being snuffed during epileptic episodes and the juice being used as a purgative. The root, on the other hand, is used to treat eye and gum disorders because of its diuretic and laxative characteristics. [8]. Merremia emarginata has resin, glycosides, reducing sugars, and starch, according to chemical studies. While the aqueous extract contains amino acids and carbohydrates, the petroleum ether extract contains lipids and fixed oil [9]. Chemical analyses of the seeds also show that caffeic, p-coumaric, ferulic, and sinapic acid esters are present [10]. These chemical components probably contribute to Merremia emarginata's medicinal qualities and may oversee its possible therapeutic benefits in the treatment of a variety of diseases.

Materials

Methods

Preparation of Ethanol Extract:

Merremia emarginata Burm. F. was obtained as a whole plant from Tirunelveli, Tamilnadu, India in November 2011 and certified by Prof. Jayaraman at PARC, Tambaram, Chennai. Fresh plant material was properly cleaned, shade dried, and then mechanically ground into a coarse powder. In a soxhlet extractor, the coarse powder was extracted with alcohol for 18 hours. Following extraction, the extract was dried in a vacuum desiccator after the solvent that was used was entirely removed by distillation. Then, the anti-inflammatory, anti-arthritic, and analgesic properties of this alcoholic extract were examined [11].

Colonies of inbred strains of Wistar albino rats and Swiss mice were purchased from the C. L. Baid

Metha College of Pharmacy for the experimental experiments. The animals were housed in typical lab settings with temperatures kept between 23 and 25 °C, a 12-hour light/dark cycle, and ad libitum access to conventional pellet diets (Hindustan Lever, Bangalore). The animals were exposed to these circumstances for a week before to the experiment. They were split up into six groups of six animals each at random. The Institute's Animal Ethics Committee oversaw the care of the animals while adhering closely to all handling principles and regulations. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), with the reference number IAEC/XXIX/10/2023.

According to the recommendations of the Organization for Economic Cooperation and Development (OECD), acute toxicity experiments were carried out using the acute oral toxic class technique in order to determine the safe dose of the extract. Two male and one female rat were each given a single administration of the EME's beginning dose of 2000 mg/kg body weight, and they were monitored for three days to look for any significant changes in body weight and toxicity indicators. For a total of fourteen days, observations were taken during a second seven-day run of the experiment using the same dose level of EME [13].

Anti-inflammatory activity

The animals were separated into five groups of six albino Wistar rats each for the purpose of evaluating anti-inflammatory activity (n=6). Sub-plantar injection of freshly made 0.1% carrageenan suspension in normal saline into the right hind paw of each rat resulted in acute paw edema (acute inflammation). Same volume of 0.1% normal saline was injected into the left hind paw. One hour before to the delivery of carrageenan, the rats received either a vehicle, EME, or indomethacin pre-treatment. A plethysmometer was used to measure the paw size in millimeters during the first and third hours following carrageenan administration. The following formula [14] was used to get the % inhibition: Percentage Inhibition = (Vc - Vt) / Vc × 100

Where, Vc = Mean increase in paw volume in the control group, Vt = Mean increase in paw volume in the test group.

Anti-arthritis activity

In order to measure the anti-arthritis activity, male rats were given a single sub-plantar injection of 0.1 ml of Complete Freund's adjuvant (CFA), which contained 1.0 mg of dry heat-killed Mycobacterium tuberculosis per milliliter of sterile paraffin oil. A digital plethysmometer was used to periodically check the edema in the hind paws. Six groups of rats were used, and various treatments were given to each group. Drug treatment began on the day of adjuvant injection (0 day), started 30 minutes beforehand, and lasted for 21 days. A plethysmometer and a vernier caliper were used to measure the paw volume and thickness on the first, fourth, eighth, fourteenth, and twenty-first days. On the appropriate days, the mean variations in the initial paw volume and the injected paw edema were calculated [15]. The animals' body weight was also determined on the first day following induction and at the end of the 21st day. For the assessment of serum parameters such as Hb, RBC, WBC, and ESR using several diagnostic kits, blood samples were obtained via retro orbital puncture under light ether anesthesia [16].

Experimental model

Four groups of six animals each were formed from the animals.

1. The control group, Group I, was given 0.5 ml of olive oil as the vehicle.
2. The arthritic control group, Group II, was made up of arthritic rats.

3. For the first 14 days, Groups III and IV were given 0.5 ml of the vehicle. From Day 15 to Day 28, they were given EME (400 mg/kg) and MFME (100 mg/kg), respectively.

The animals were decapitated in the cervical region and killed on day 29. Blood was drawn both with and without EDTA in order to separate the plasma from the serum. Acute phase proteins including CRP, ceruloplasmin (CP), and fibrinogen as well as immunoglobulins like IgG, IgM, and IgA were estimated using the blood samples that were taken. IgM and IgA were quantified in rat serum by turbidometry immunoassay, whereas IgG was assessed quantitatively by the coagulation of gamma globulin using glutaraldehyde reagent.

An ice-cold saline solution was used to cool the liver and kidney after they had been separated, cleaned, and placed in it. Weighed amounts of tissues were homogenized in 0.1M Tris-HCl buffer, pH 7.4, to create 10% tissue homogenates from the organs. In plasma, liver, and kidney homogenates, lysosomal enzymes like acid phosphatase (ACP), d-glucuronidase, d-galactosidase, cathepsin D, and N-acetyl-d-glucosaminidase were quantified. Additionally, glycoproteins with protein-bound carbohydrates were identified in plasma and urine samples [17,18]. Additionally, joint extract was made in order to study proinflammatory cytokines.

Insights into the effects of EME and MFME on acute phase proteins, immunoglobulins, lysosomal enzymes, and protein-bound carbohydrate components in various tissues were gained thanks to the measurement of numerous parameters made possible by this extensive experimental setup. To further our understanding of the processes behind the anti-arthritic actions of Merremia emarginata extracts, valuable information about the potential modulation of proinflammatory cytokines was provided by the analysis of joint extract [19,20].

Estimation of protein bound carbohydrates in plasma and urine

In this investigation, plasma-based glycoproteins were first precipitated and subsequently hydrolyzed. 0.05 ml of plasma and 2.0 ml of alcohol were combined before being centrifuged to start the procedure. The supernatant and precipitate were separated by centrifugation. Carefully decanting the supernatant left behind the precipitate, which was then hydrolyzed with acid. The protein-bound carbohydrates were released during this hydrolysis process and could now be quantitatively estimated[21]. The estimation of particular protein-bound carbohydrates in the plasma samples, including their composition and quantity, was greatly helped by the estimation of hexose, hexosamine, hexuronic acid, fucose, and sialic acid. Additionally, the experimental patients' 24-hour urine samples were taken and examined. Analysis was done on the urine samples to determine whether hexosamine, hexuronic acid, and hydroxyproline were present. These analyses sought to ascertain a compound's excretion levels in order to shed light on its metabolic processing and relevance to the research parameters[22].

Preparation of joint extract

The inflammatory paw tissues in this investigation were surgically removed, and the paws were severed 0.5 cm above the ankle. To get rid of any dirt or pollutants, these removed tissues were then extensively washed in ordinary saline. To retain the tissues' cellular components and stop deterioration, they were then quickly frozen in liquid nitrogen. The frozen joints were ground into a fine powder under liquid nitrogen using a mortar and pestle to prepare the tissue samples for further investigation. Exactly 2 ml of sterile saline solution was then added to a glass homogenizer with the pulverized tissue to generate a suspension. To ensuring uniform distribution and destroying cell structures, the tissue slurry was manually homogenized for 2 minutes. Centrifugation was

then performed on the suspension for 20 minutes at 1500 g and 4 °C. This procedure sought to separate the liquid supernatant from the cellular waste and other solid components. After the initial centrifugation, the resulting supernatant underwent a second round of centrifugation for 10 minutes at 3000 g. Any lingering solid particles were removed from the supernatant during this extra centrifugation step [23]. The resultant supernatant, which contained the soluble parts of the tissue sample, was then painstakingly gathered and aliquoted. To maintain the integrity of the protein content, the aliquots were then chilled at a very low temperature of 70 °C.

Assay of proinflammatory cytokines TNF-α and IL-1β by enzyme linked immunosorbent assay (ELISA)

The enzyme-linked immunosorbent assay (ELISA) technique was used to determine the concentrations of the proinflammatory cytokines TNF- and IL-1. For this investigation, a modified version of Sharma and Singh's methodology was used.First, 100 l of joint extract that had been diluted with carbonate buffer and contained 50 g of protein was applied to the wells of a microtitre plate. After that, the plate was kept at 4 °C overnight to allow the extract's proteins to cling to the wells [24,25]. The plate was cleaned three times using a washing solution after incubation to get rid of any unbound or improperly bound components. The wells were then filled with 200 l of a blocking solution and incubated at 37 °C for 1 hour in order to remove any nonspecific binding sites. By inhibiting the succeeding antibodies, it was made sure that they would only bind exclusively to the target cytokines. Following the blocking procedure, 100 l of primary antibodies were added to the wells at a dilution of 1:5000, specifically rabbit monoclonal antibodies against TNF- and IL-1. Following that, the plate was incubated for 1 hour at 37 °C to allow the antibodies to bind precisely to their cytokine targets. The plate was again washed to eliminate any unbound antibodies following the incubation with the primary antibodies. Then, 100 l of secondary antibodies were diluted 1:500 and were goat anti-rabbit antibodies IgG-horseradish peroxidase (HRP) conjugated. These secondary antibodies would join the primary antibodies, creating a visible complex that could be detected.To aid in the secondary antibodies' ability to attach to the primary antibodies, the plate was once more incubated at 37 °C for an hour. The plate was then rinsed to get rid of any secondary antibodies that weren't bound after this incubation. 100 l of the o-phenylenediamine (OPD) reagent were added to the wells to measure the bound HRP activity. The secondary antibody conjugate's HRP enzyme would work with the OPD to catalyze a color-producing reaction.To enable the color to develop, the plate was incubated for 20 minutes at room temperature in the dark. Each well received 50 l of a stopping solution to halt the reaction. Finally, a microplate reader was used to detect the samples' absorbance at 492 nm. The amounts of TNF- and IL-1 in the joint extract samples were quantified using the absorbance values, revealing important details about the inflammatory response in the examined arthritis model..

Immunoassay of proinflammatory cytokines TNF-α and IL-β

The sample solubilizing buffer (SSB) was applied to the 50 g of protein in each of the protein samples before boiling them for 5 minutes. They were then divided using 10% SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis).Using an electric field at 30 V for three hours, the separated proteins on the gel were then transferred onto a nitrocellulose membrane (Hybond C+, Amersham Life Sciences).The membrane was washed three times with phosphate-buffered saline (PBS) to guarantee optimal protein detection, and then blocked using TBST buffer (20 mM Tris, 500 mM NaCl, 0.1% Tween 20, pH 7.5) containing 5%

non-fat dried milk. After blocking, rabbit monoclonal anti-TNF- and IL-1 antibodies were diluted at a ratio of 1:5000 in TBST buffer containing 1% non-fat dry milk and incubated with the membrane. For three hours, this incubation was gently conducted at ambient temperature.

The blots were three times for five minutes with TBST buffer after the primary antibody incubation. The membrane was then exposed to goat anti-rabbit or rabbit anti-goat HRP-conjugated secondary antibodies (goat anti-rabbit antibody IgG-HRP conjugated) diluted at a ratio of 1:500 in phosphate-free TBST buffer containing 5% non-fat dried milk for an additional 75 minutes at room temperature. This technique provided valuable insights into the inflammatory response and the efficacy of the test compounds in modulating cytokine levels [26].

Statistical analysis

Significance of the difference between mean values were determined by one-way analysis of variance (ANOVA) followed by the Tukey's test for multiple comparison using Graph pad prism [27]. Significant difference between control and treatment groups was assigned at $p < 0.05$.

RESULTS

Table 1: Effect of EME & MFME on Haematological parameter in RA (Molecular level)

Treatment	Hb Count (g/dl)	RBC Count ($x10^6$/ mm^3)	WBC Count ($x10^3/mm^3$)	ESR (mm/hr)	RF (IU/ml)
Control	14.33 ± 0.36	5.23 ± 0.84	7.85 ± 0.74	3.17 ± 0.85	25 ± 3.35
Negative Control	9.72* ± 0.88	3.13* ± 0.81	12.37* ± 1.24	6.73* ± 1.14	65* ± 4.66
EME (400mg/kg)	12.62* ± 1.12	5.28* ± 0.12	8.13* ± 1.14	3.25* ± 0.83	26* ± 2.75
MFME (100mg/kg)	13.72* ± 1.35	5.65* ± 0.97	7.95* ± 0.83	3.37* ± 1.12	28* ± 3.44

Values are expressed as mean ± S.E.M. (n=6), * P < 0.05. Data were analysed by using one-way Anova followed by Tukey's multiple comparison test.

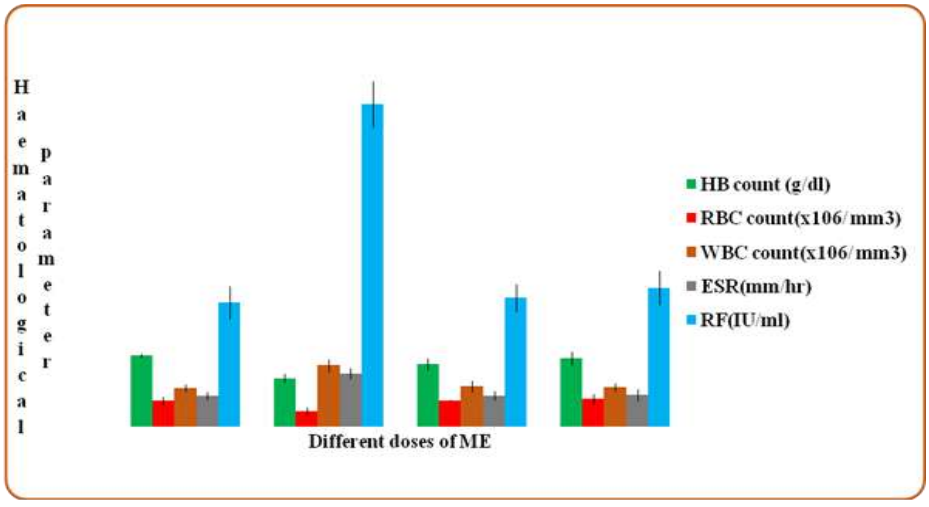

Figure 1: Different dose of ME Vs Haematological parameter (molecular level)

Table 2: Effect of EME & MFME on Acute Phase Proteins in RA (Molecular level)

Treatment	CRP (mg/dl)	Ceruloplasmin(mg/dl)	Fibrinogen(mg/dl)
Control	0.58 ± 0.09	3.78 ± 0.78	224.67 ± 5.65
Negative Control	0.97* ± 0.15	7.23* ± 1.15	512.56* ± 8.76
EME	0.56* ± 0.12	3.86* ± 0.87	235.89* ± 9.43

(400mg/kg)			
MFME (100mg/kg)	0.62* ± 0.24	4.20* ± 0.60	289.78* ± 7.35

Values are expressed as mean ± S.E.M. (n=6), * P < 0.05. Data were analysed by using one-wayAnova followed by Tukey's multiple comparison test.

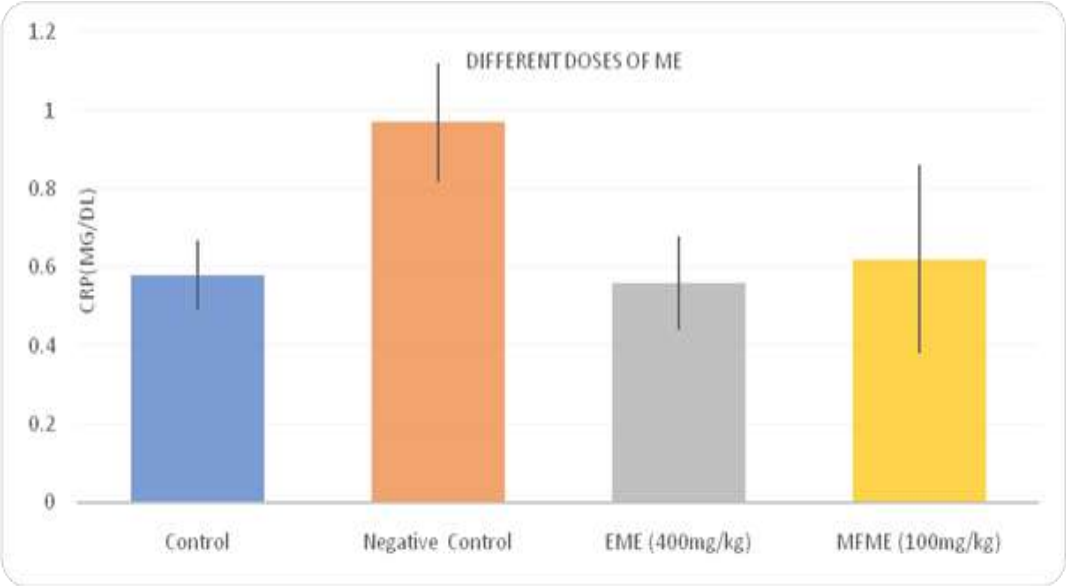

Figure 2: Different doses of ME Vs CRP

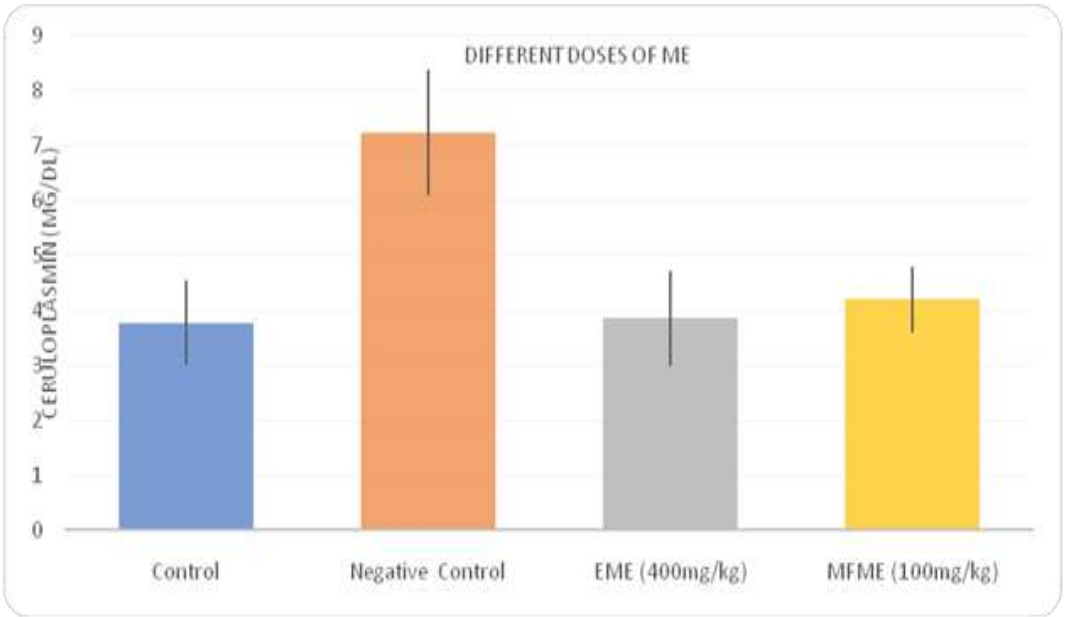

Figure 2.1: Different doses of ME Vs Ceruloplasmin

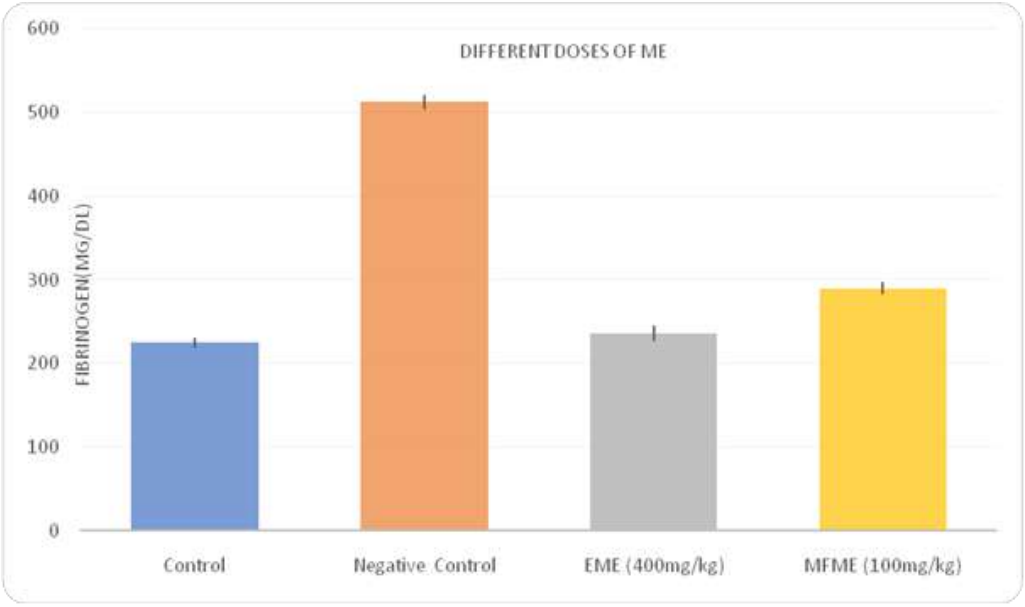

Figure 2.2: Different doses of ME Vs Fibrinogen

Table 3: Effect of EME & MFME on the levels of Immunoglobulins in RA (Molecular level)

Treatment	IgG (mg/dl)	IgA (mg/dl)	IgM (mg/dl)
Control	268.34 ± 26.56	212.34 ± 19.24	92.78 ± 9.67
Negative Control	389.56* ± 28.46	310.09* ± 23.10	198.67* ± 17.73
EME (400mg/kg)	274.34* ± 27.76	228.20* ± 20.33	94.40* ± 10.32
MFME (100mg/kg)	302.67* ± 26.12	298.88* ± 21.87	108.44* ± 11.75

Values are pressed as mean ± S.E.M. (n=6), * P < 0.05. Data were analysed by using one way Anova followed by Tukey's multiple comparison test.

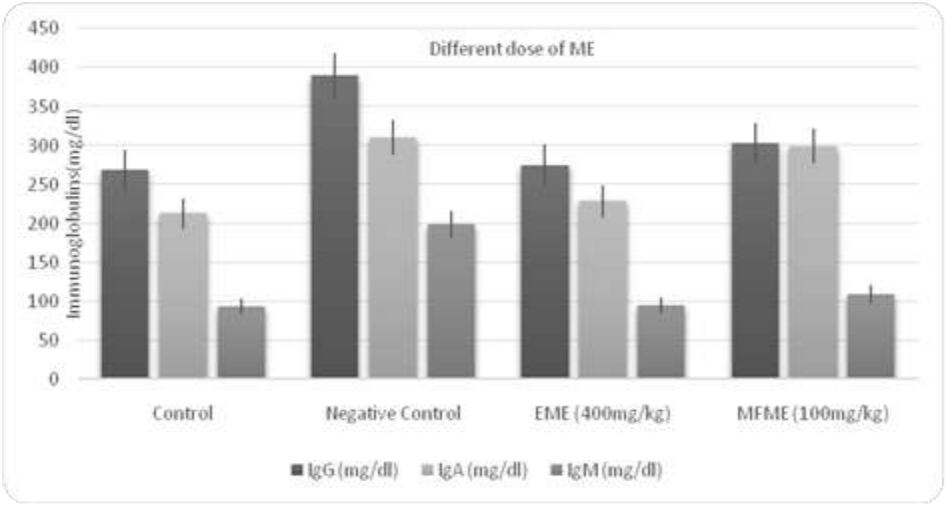

Figure 3: Different doses of ME Vs Immunoglobulins

Table 4: Effect of EMEon the inflammatory mediators in RA (Molecular level)

Treatment	TNF-α	IL-1β
Control	100	100
Negative Control	234* ± 1.45	224* ± 2.37
EME (400mg/kg)	124* ± 2.56	115* ± 1.75
MFME (100mg/kg)	135*± 1.80	142* ± 1.65

Values are expressed as mean ± S.E.M. (n=6), * P < 0.05. Data were analysed by using one-wayAnova followed by Tukey's multiple comparison test

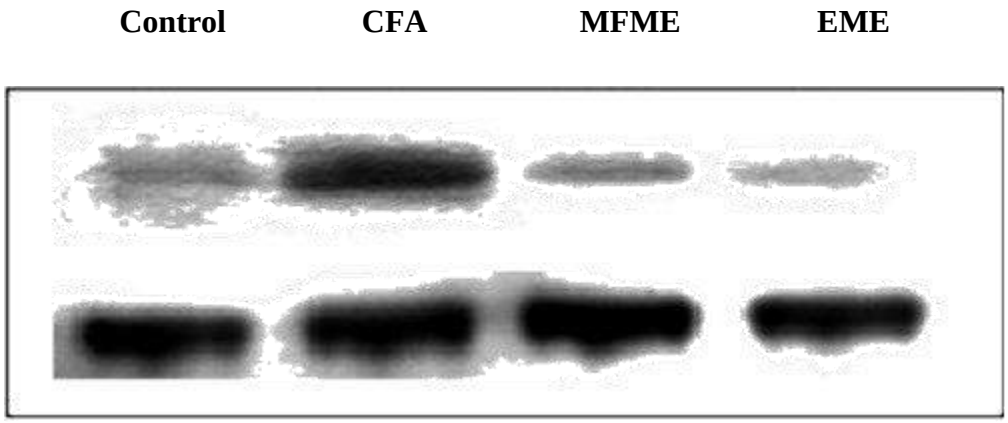

Figure 4 (a): Gel electrophoresis TNF alpha

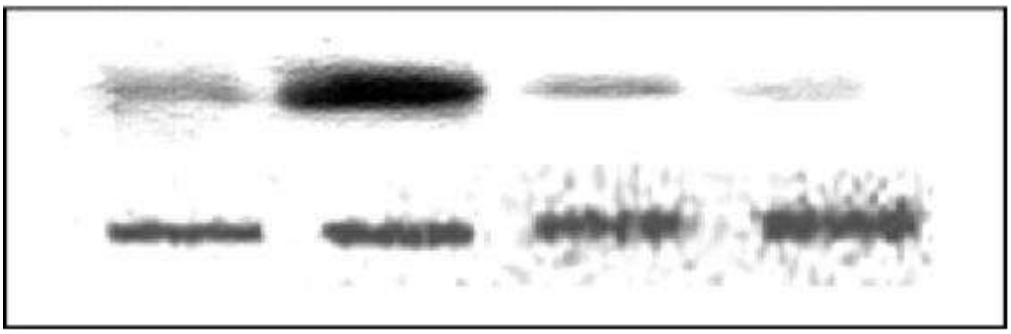

Figure 4.1 (a): Gel electrophoresis IL 1β

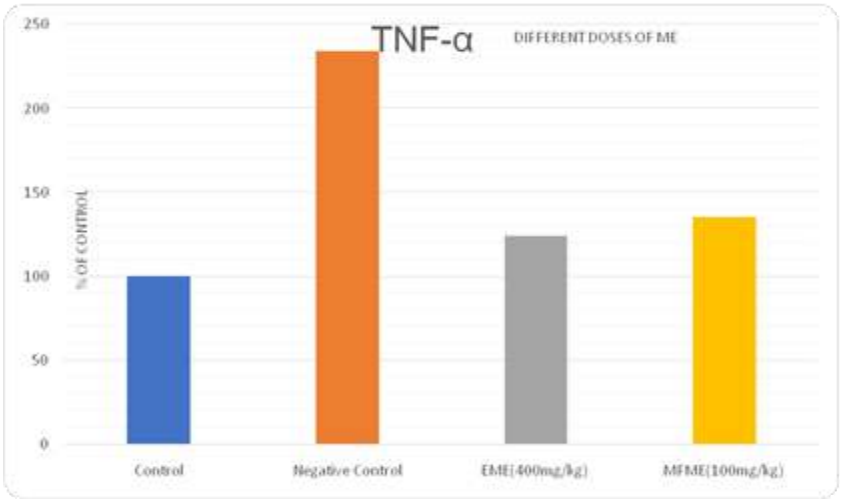

Fig 4 (b) Different doses of ME Vs % of Control of TNF- α

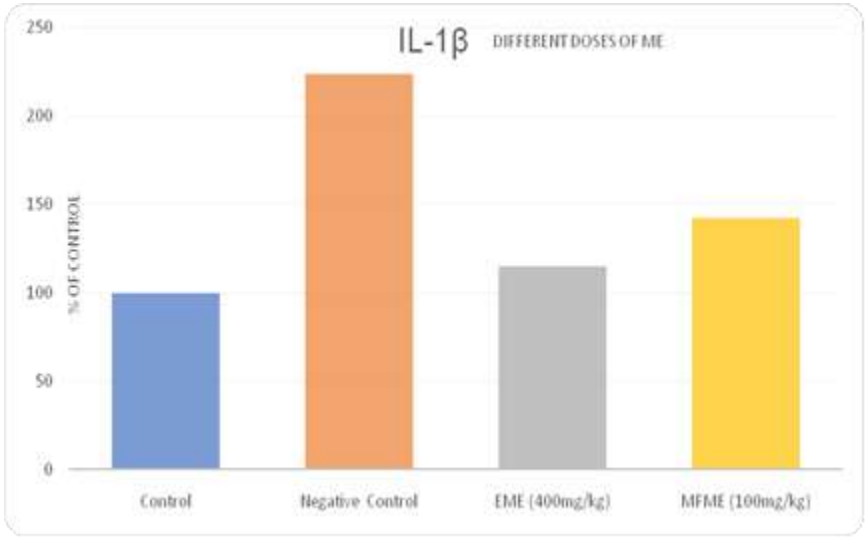

Fig 4.1 (b): Different doses of ME Vs % of control of IL-1β

Table 5: Effect of EME & MFME on plasma lysosomal enzymes in RA (Molecular level)

Treatment	Acid Phosphatase (ACP)	β-D-Glucuronidase	β-D-Galactosidase	Cathepsin D	N-Acetyl-β-D-Glucosaminidase
Control	1.98 ± 0.45	2.74 ± 0.79	1.48 ± 0.26	8.62 ± 1.24	2.45 ± 0.27
Negative Control	4.25* ± 0.78	17.28* ± 1.10	3.67* ± 0.98	18.67* ± 1.45	3.89* ± 0.56
EME (400mg/kg)	2.10* ± 0.85	3.12* ± 0.94	1.65* ± 0.34	9.04* ± 0.98	2.38* ± 0.46
MFME (100mg/kg)	2.45* ± 0.39	3.98* ± 0.57	1.98* ± 0.25	9.79* ± 1.14	2.78* ± 0.49

Values are expressed as mean ± SD, for 6 animals, Units: Acid Phosphatase (ACP) × 10^{-2} µmol of phenol formed/min/mg protein, β-D-Glucuronidase, β-D-Galactosidase &N-Acetyl-β-D-Glucosaminidase × 10^{-2} µmol of p-nitrophenol formed/h/mg protein, Cathepsin D× 10^{-3} µmol of tyrosine liberated/h/mg/protein, statistical significance at $P < 0.05$*

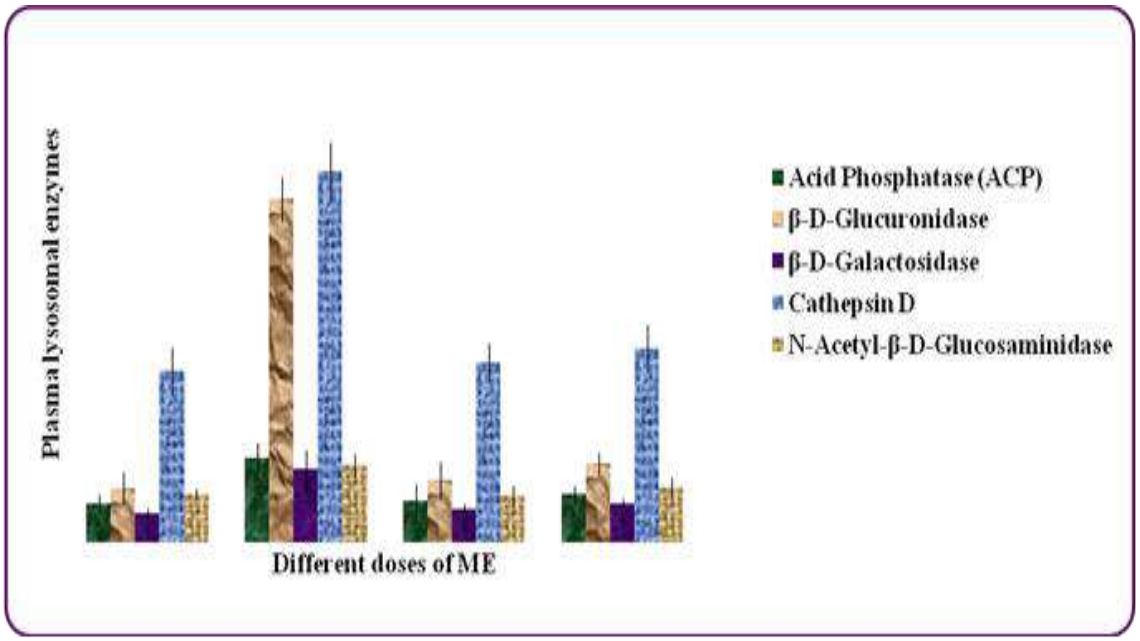

Figure 5: Different doses of ME Vs Plasma lysosomal enzymes

Table 6: Effect of EME & MFME on liver lysosomal enzymes in RA (Molecular level)

Treatment	Acid Phosphatase (ACP)	β-D-Glucuronidase	β-D-Galactosidase	Cathepsin D	N-Acetyl-β-D-Glucosaminidase
Control	8.78 ± 0.49	34.89 ± 0.86	8.62 ± 0.76	32.50 ± 0.56	30.46 ± 0.56
Negative Control	24.44* ± 1.45	49.08* ± 1.76	17.86* ± 1.67	70.89* ± 1.89	65.58* ± 1.78
EME (400mg/kg)	8.90* ± 0.97	33.32* ± 1.23	8.46* ± 0.78	30.24* ± 1.25	29.04* ± 1.59
MFME (100mg/kg)	9.27* ± 0.98	37.90* ± 1.45	9.23* ± 0.90	37.04* ± 1.87	32.79* ± 1.45

Values are expressed as mean ± S.D, for 6 animals, Units: Acid Phosphatase (ACP) × 10^{-2} µmol of phenol formed/min/mg protein, β-D-Glucuronidase, β-D-Galactosidase &N-Acetyl-β-D-Glucosaminidase × 10^{-2} µmol of p-nitrophenol formed/h/mg protein, Cathepsin D× 10^{-3} µmol of tyrosine liberated/h/mg/protein, Statistical significance at $P < 0.05$*

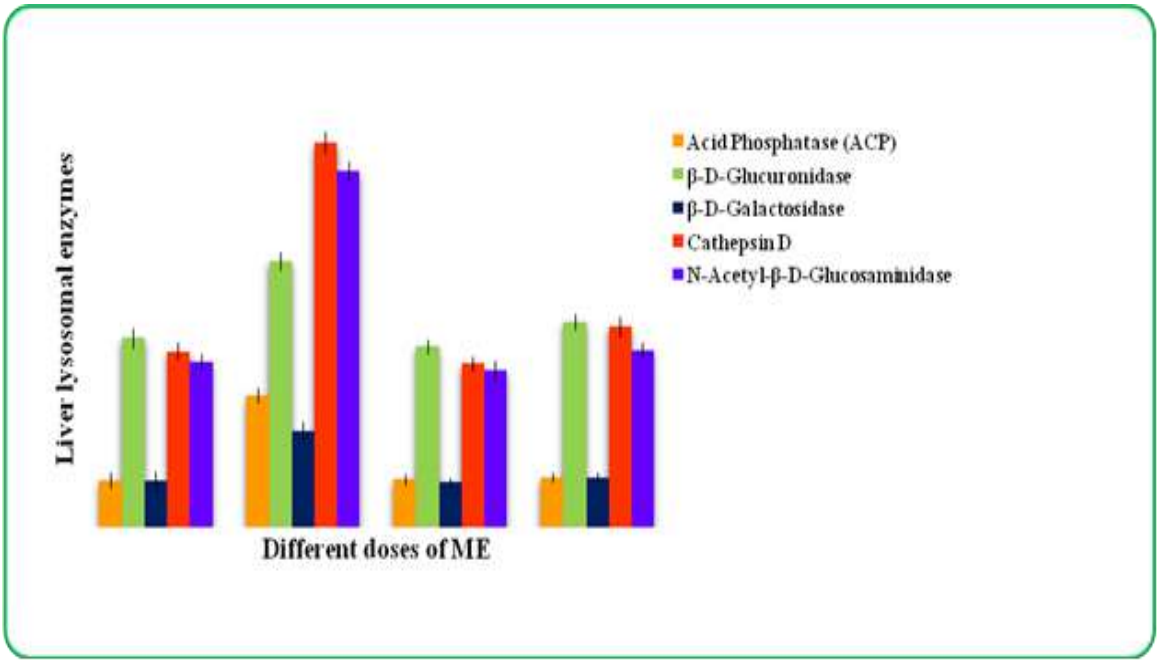

Figure 6: Different doses of ME Vs Liver lysosomal enzymes

Table 7: Effect of EME & MFME on kidney lysosomal enzymes in RA (Molecular level)

Treatment	Acid Phosphatase (ACP)	β-D-Glucuronidase	β-D-Galactosidase	Cathepsin D	*N*-Acetyl-β-D-Glucosaminidase
Control	3.46 ± 0.57	22.78 ± 0.70	14.78 ± 0.48	47.24 ± 0.78	46.19 ± 0.56
Negative Control	9.67* ± 0.89	44.09* ± 1.67	28.65* ± 1.45	72.45* ± 1.67	65.87* ± 1.30
EME (400mg/kg)	3.24* ± 0.35	23.14* ± 1.18	15.12* ± 1.04	46.87* ± 1.47	47.27* ± 1.38
MFME (100mg/kg)	3.98* ± 0.46	23.67* ± 1.07	16.04* ± 1.10	48.20* ± 1.20	48.36* ± 1.76

Values are expressed as mean ± SD, for 6 animals, Units: Acid Phosphatase (ACP) × 10^{-2} µmol of phenol formed/min/mg protein, β-D-Glucuronidase, β-D-Galactosidase &N-Acetyl-β-D-Glucosaminidase × 10^{-2} µmol of p-nitrophenol formed/h/mg protein, Cathepsin D× 10^{-3} µmol of tyrosine liberated/h/mg/protein, statistical significance at $P < 0.05$*

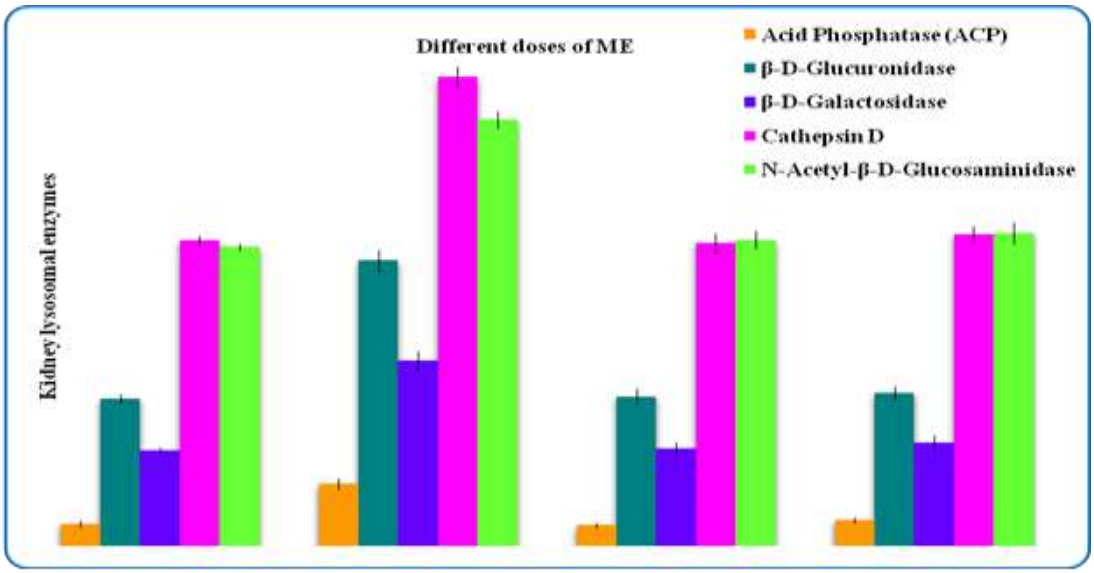

Figure 7: Different doses of ME Vs Kidney lysosomal enzymes

Table 8: Effect of EME & MFME on plasma protein bound carbohydrates in RA (Molecular level)

Treatment	Hexose	Hexosamine	Hexuronic acid	Fucose	Sialic acid
Control	127.43 ± 0.89	39.24 ± 0.57	61.35± 0.78	12.23 ± 0.35	52.65 ± 0.87
Negative Control	198.67* ± 2.45	62.47* ± 1.39	109.56*± 1.78	19.09* ± 0.89	84.37* ± 1.59
EME 400mg/kg	128.54* ± 2.30	39.78* ± 1.27	61.98*± 1.60	12.56* ± 0.87	52.98* ± 1.48
MFME 100mg/kg	129.23* ± 2.87	40.59* ± 1.39	62.46*± 1.48	13.45* ± 0.79	53.57* ± 1.67

Values are expressed as mean ± S.E.M. (n=6), * $P < 0.05$. Data were analysed by using one way Anova followed by Tukey's multiple comparison test

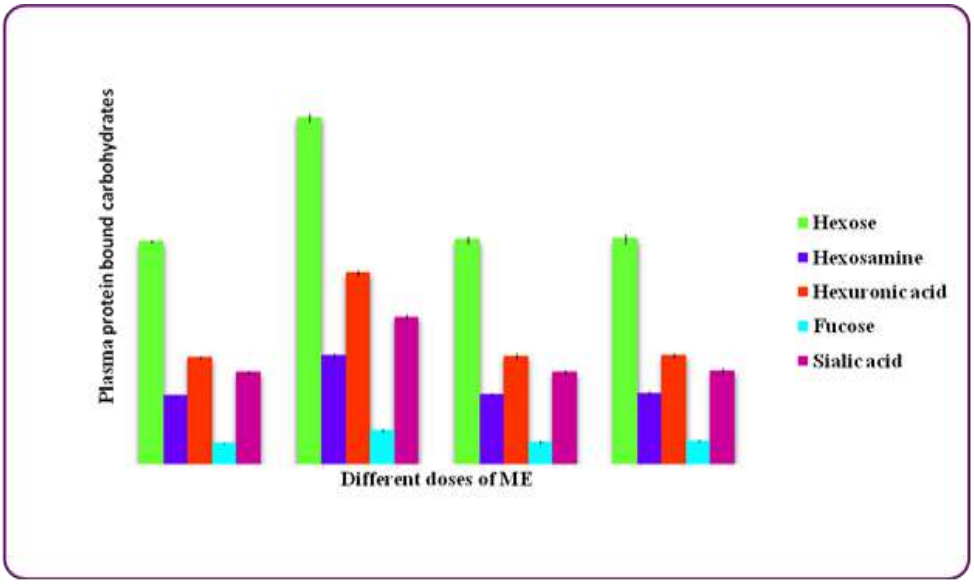

Figure 8: Different doses of ME Vs Plasma protein bound carbohydrates

Table 9: Effect of EME & MFME on the level's urinary collagen degradative products in RA (Molecular level)

Treatment	Hexosamine (mg/100mg creatinine)	Hexouronic acid (mg/100mg creatinine)	Hydroxyproline (mg/100mg creatinine)	Total GAGS (mg/24hr urine)
Control	74.43 ± 1.09	12.35 ± 0.88	6.85 ± 0.78	5.23 ± 0.35
Negative Control	148.67* ± 2.45	22.47* ± 1.39	10.56* ± 1.12	9.09* ± 1.11
EME (400mg/kg)	88.54* ± 2.30	14.78* ± 1.27	7.98* ± 1.20	6.56* ± 0.97
MFME (100mg/kg)	79.23* ± 2.87	15.29* ± 0.92	8.16* ± 1.28	7.05* ± 1.19

Values are expressed as mean ± S.E.M. (n=6), * P < 0.05. Data were analysed by using one-way Anova followed by Tukey's multiple comparison test

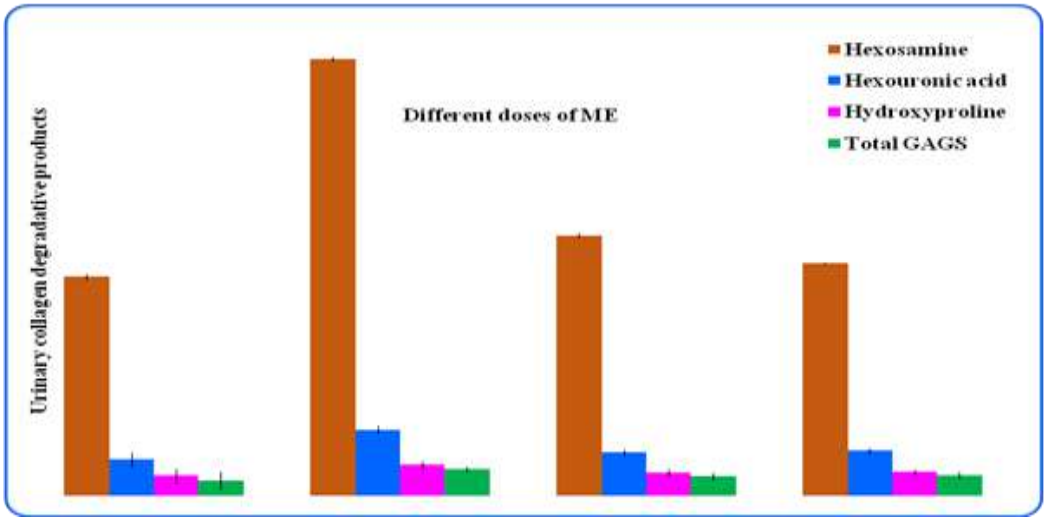

Figure 9: Different doses of ME Vs Urinary collagen degradative protein

A) Control

B) Negative control

C) EME (400mg/kg)

D) MFME(100mg/kg)

Figure 10 : Radiographic analysis of molecular studies

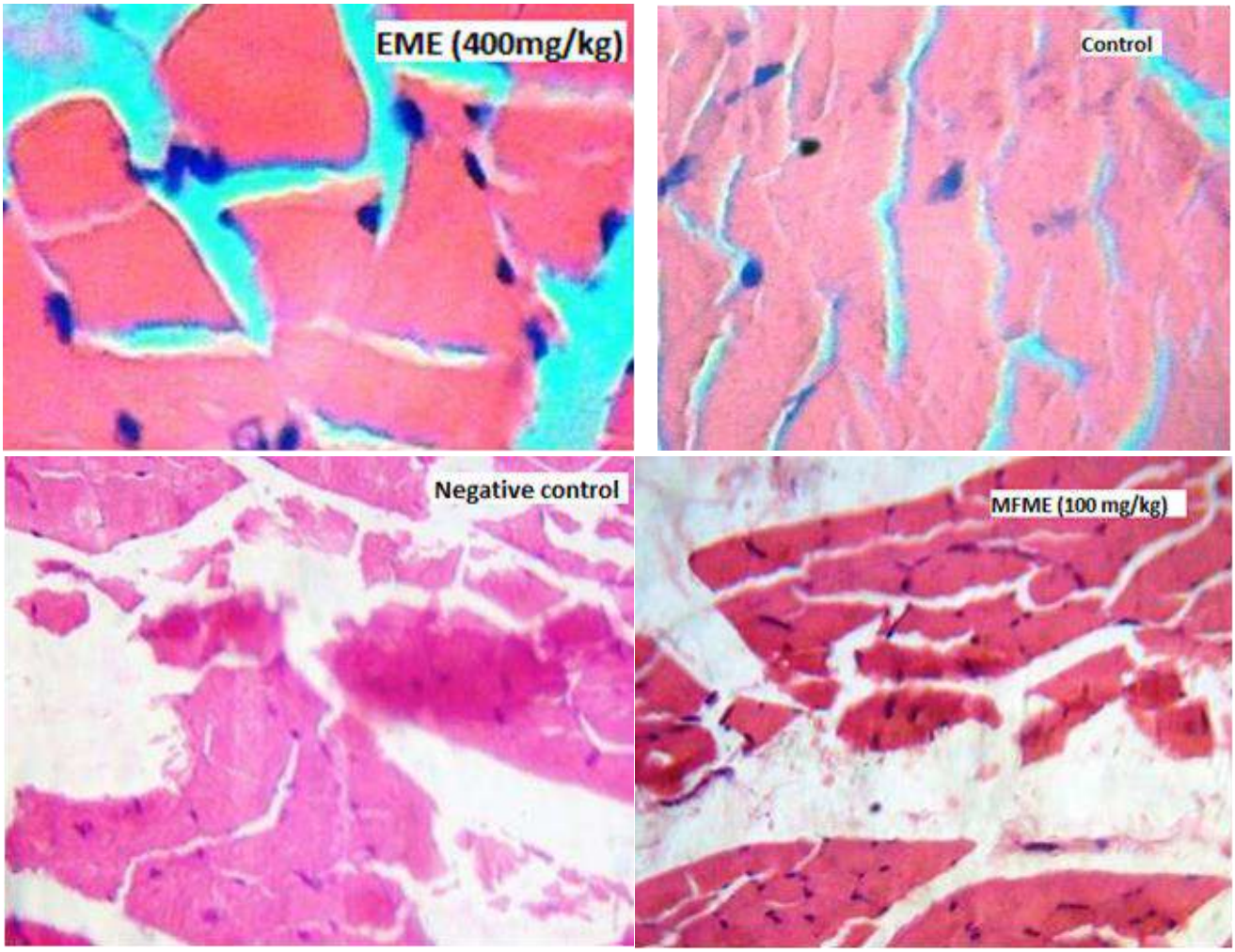

Figure 11: Histopathology Of Molecular Studies

DISCUSSION

According to extensive biological research, Merremia emarginata clearly shown exceptional anti-cancer and anti-arthritic capabilities. Merremia emarginata's alcoholic extract (EME) and methanol fraction (MFME) were put through in vivo anti-arthritic investigations employing a CFA-induced model in order to further validate these findings. The significance and effectiveness of the treatments were evaluated using proper statistical methods after the results from these investigations were assessed. Haematological variables including Hb and RBC were lower and WBC, ESR, and RF levels were higher in the arthritic state. EME (400 mg/kg) and MFME (100 mg/kg) therapy, however, considerably reversed these abnormalities and returned the levels to normal. Notably, compared to MFME, EME (400mg/kg) showed a more significant and severe effect. (Figure 1 and Table 1). Acute phase proteins such CRP, ceruloplasmin, and fibrinogen were found in higher concentrations in CFA rats. EME (400 mg/kg) and MFME (100 mg/kg) therapy, however, markedly decreased these protein levels and brought them closer to normal ranges. In contrast to MFME (100mg/kg), EME (400mg/kg) therapy shown a more pronounced reduction in acute phase proteins. Figures 2.1, 2.2, and 2.3 from Table 2

Immunoglobulin levels (IgG, IgA, and IgM) significantly increased in CFA rats. Nevertheless, treatment with EME (400 mg/kg) and MFME (100

mg/kg) led to a marked decline in immunoglobulin levels. In contrast to MFME (100mg/kg), EME (400mg/kg) shown a more pronounced decrease in immunoglobulin levels. Figure 3 and Table 3

TNF- and IL-1 protein expression was significantly higher in CFA rats compared to the animals in the control group. However, treatment with EME (400 mg/kg) and MFME (100 mg/kg) resulted in a notable reduction in TNF- and IL-1 protein expression. Notably, when compared to MFME (100mg/kg), EME (400mg/kg) showed a much lower expression of these proteins. (Figures 4.1 and 4.2, as well as Table 4). The results of the study demonstrated that the alcoholic extract (400mg/kg) and methanolic fraction (100mg/kg) of ME significantly reduced the activity of lysosomal enzymes, including glycohydrolases such as Acid Phosphatase (ACP), β-D-Glucuronidase, β-D-Galactosidase, Cathepsin D, and N-Acetyl-β-D-Glucosaminidase in plasma, liver, and kidney of the experimental animals. These lysosomal enzyme levels were elevated in the arthritic model caused by CFA, and treatment with EME (400 mg/kg) led to a more significant decline in the activity of these enzymes than MFME (100 mg/kg). (Figures 6 and 7, Tables 6 and 7). In the plasma of test animals, levels of collagen breakdown products such hexose, hexosamine, hexuronic acid, fucose, and sialic acid were significantly reduced by the ethanolic extract (400 mg/kg) and methanolic fraction (100 mg/kg) of ME. The levels of plasma protein-bound carbohydrates were significantly higher in the CFA-induced arthritis model than in the control mice. Notably, compared to MFME (100mg/kg), the therapy with EME (400mg/kg) had a more noticeable effect on lowering the collagen degradative products in plasma. Figure 8 and Table 8

Total GAGs and their breakdown products, such as hexosamine, hexuronic acid, and hydroxyproline, were significantly excreted in greater amounts in the urine of CFA rats. However, the use of EME (400 mg/kg) and MFME (100 mg/kg) reduced the amount of these collagen-degrading substances in urine significantly. In contrast to MFME (100mg/kg), EME (400mg/kg) therapy significantly reduced these collagen-degradative compounds in urine. (See Figure 9 and Table 9). The phalangeal region of the joints of CFA-induced rats showed diffuse joint involvement, decreased joint space, and soft tissue edema. However, EME (400 mg/kg) and MFME (100 mg/kg) therapy reduced soft tissue edema but did not cause the joint space to become smaller. (Figure10) Inflammation was seen in the infiltration of mononuclear cells and the growth of synovial tissue in rats exposed to CFA. However, there was no inflammation in the groups who received EME (400mg/kg) and MFME (100mg/kg) treatments.(Figure11)

Molecular effect of *Merremiaemarginata*Burm.f on the inflammatory mediators TNF-α and IL-1β

Based on the aforementioned scientific research, Merremia emarginata extracts showed strong effectiveness against rheumatoid arthritis and cancer. As a result, more research on Merremia emarginata was done at the molecular level.

Role in restoration of Haematological parameters (Table 1 and Figure 1)

Anemia is present in arthritic rats, as evidenced by the drop in Hb and RBC counts that was seen in these animals. Rheumatoid Arthritis (RA) frequently manifests as anemia, which is characterized by a mild hypochromic and normocytic state and a slight decrease in mean corpuscular hemoglobin concentration[28]. This anemia may be caused by lower plasma iron levels brought on by iron sequestration in the reticuloendothelial system and synovial tissue, which prevents the bone marrow from responding to the anemic condition as it should. . The action of IL-1 in conjunction with the acute phase

response is thought to have caused the drop in plasma iron. Therefore, it is conceivable to speculate that endothelial cells in the spleen sequester less deformable erythrocytes, which ultimately shortens their half-life and causes anemia in adjuvant arthritis. The immune system being stimulated by the presence of the foreign body, CFA, may be the cause of the observed increase in WBC and platelet counts. The presence of inflammatory mononuclear cells in the joints of CFA-induced rats supports this immune response [29]. In order to assess the acute phase response and assess the disease activity in rheumatoid arthritis (RA), the increased ESR (Erythrocyte Sedimentation Rate) is used. While ESR is influenced by a number of variables, including plasma concentrations of fibrinogen, immunoglobulins, RF, and Hb, it provides a comprehensive reflection of the disease status, whereas CRP (C-Reactive Protein) is thought to be a more specific marker for inflammation.

Role in modulation of acute phase proteins (Table 2 and Figures 2.1, 2.2 and 2.3)

In CFA-induced rats, the acute phase response has proven to be a useful method for assessing the anti-inflammatory effects of various medications. A catabolic and damaging state in the body is reliably indicated by elevated acute phase proteins. The elevated activation of proinflammatory cytokines including IL-1, TNF-, and IL-6 is principally responsible for the aberrant rise in plasma CRP levels. Additionally, the vascular epithelium is activated by the CRP promoter genes, hastening the blood's acute phase reaction. Ceruloplasmin, a protein with multiple biological functions, is linked to altered copper metabolism in RA[30]. Documented increases in the blood levels of ceruloplasmin and copper serve as proof of this transformation. This rise has been reported, and it might be attributed to the overexpression of IL-6 in arthritic animals. Fibrinogen has a long half-life of about 305 days, which makes it important for tracking chronic inflammation. The observed rise in plasma fibrinogen levels and its deposition as fibrin in synovial fluid firmly support its critical role in the persistence of various inflammatory joint disorders. Fibrinogen thus acts as an indication with an inverse relationship to anti-inflammatory medication, emphasizing its usefulness in gauging the severity and evolution of such inflammatory disorders.

Role in immunomodulation

The raised levels of IgG, IgA, and IgM in CFA rats may be explained by the increased susceptibility to immunological stimuli and the onset of autoimmune and delayed hypersensitivity symptoms, which are indicative of RA. The generation of RF is another sign of RA[31,32]. By assisting in the activation of synovial macrophages, fibroblasts, and granulocytes in the synovial tissue as well as granulocytes in the synovial fluid, both IgA-RF and IgG-RF most likely play a crucial role in the pathogenesis of RA. The inflammatory processes seen in the joints of arthritic rats are thought to be significantly influenced by these immunological variables.

Role in modulation of inflammatory mediators (Table 4 and Figures 4.1 and 4.2)

The onset of arthritis in CFA-induced arthritis is rapid and partially reliant on TNF- and IL-1. Cytokine-based therapies, like the one utilizing EME, have been used to treat RA and have demonstrated promise in halting the development of chronic arthritis. Both experimental arthritis and rheumatoid arthritis have been found to benefit by blocking these proinflammatory cytokines.Given that macrophages are the primary cells responsible for creating these pro-destructive cytokines, the higher protein levels of both TNF- and IL-1 in CFA rats may be explained by the cytokine-mediated activation of these cells in the joints. In CFA rats, the increased production of TNF- and IL-1 is a key factor in the development and maintenance of RA. In animal models of RA, these

pro-inflammatory cytokines cause inflammatory cells like macrophages, fibroblasts like synoviocytes, and T-lymphocytes to develop into invasive, aggressive cells that resemble tumor cells.Therefore, the increased production of these cytokines may make it easier for inflammatory cells to invade the joints, resulting in the development of pannus. Additionally, the increased levels of IL-1 and TNF- in the joints of CFA rats interact synergistically, which may cause erosions by accelerating the deterioration of bone and cartilage. These findings provide important insights into possible treatment targets and emphasize the important roles of TNF- and IL-1 in the pathophysiology of RA.

Role in protective effect on lysosomal enzymes (Table 5 and Figure 5)

A change in the membrane composition of the group of cytoplasmic organelles known as lysosomes may improve their capacity to fuse with the cell membrane and discharge their contents. The structural macromolecules of connective tissue and proteoglycans, which are the essential elements of joint cartilage, synovial fluid, and soft tissues, are subsequently destroyed as a result of this. Glycohydrolase activity was seen to significantly increase in CFA rats..

Recent research has shown a substantial correlation between RA and specific glycosidases' activities, particularly those of -D-Glucuronidase and N-acetyl -D-Glucosaminidase, which are both shown to be highly active in stimulated chondrocyte supernatants and RA sera. Serum -D-Glucuronidase concentrations that are elevated have been proposed as a measure of intra-articular macrophage activity. Along with glycohydrolases, it was discovered that CFA rats had enhanced aspartyl protease and cathepsin-D activity. Cathepsin-D's proteolytic activity may be increased by pathological events, such as the proteolytic cleavage of significant cartilage extracellular matrix constituents in RA (Tables 6 and 7 and Figures 6 and 7). These results show that lysosomal enzymes are involved in the pathophysiology of RA, and that increased activity of these enzymes causes tissue injury and degradation, especially in the joints[33]. Understanding how lysosomal enzymes function in the disease process may provide new opportunities for treating RA and managing its symptoms.The increased glycohydrolase levels in CFA rats imply that the extrusion or leaking of lysosomal enzymes is a direct result of increased endocytic activity. This happens because the immunological complexes that are produced cause the leukocyte-attracting chemotactic factors to be released. Following their phagocytization of the immune complexes, these leukocytes release lysosomal enzymes that damage the cartilage and other tissues in RA. The significant rise in these glycohydrolases' activities supports the idea that CFA rats have more fragile lysosomes, which is linked to the worst joint destruction in these animals. In arthritic rats, the increased activity of these lysosomal enzymes may cause proteoglycan breakdown and subsequent collagen loss.Hyaluronic acid, a significant glycosaminoglycan, is a major component of the synovial fluid and articular surface, and its breakdown can exacerbate joint injury in RA.Surprisingly, the radiographic and histological abnormalities were successfully reversed to close to normal following treatment with EME (400mg/kg) and MFME (100mg/kg). This suggests that the joint degradation and tissue damage brought on by CFA-induced arthritis may be lessened by these plant extracts. (See Figures 10 and 11).

CONCLUSIONS

Based on the results, it can be said that lysosomal enzyme release is effectively decreased by EME (400 mg/kg) and MFME (100 mg/kg), which results in less breakdown of cartilage, bone, and synovial tissue. As a result, this encourages the

production of glycosaminoglycans (GAGs), which are crucial structural elements for maintaining joint health. Additionally, these plant extracts show an improvement in the antioxidant system, which helps to lessen oxidative stress, a characteristic of inflammatory disorders. Additionally, proinflammatory cytokines TNF- and IL-1, which are important participants in the inflammatory process, are shown to be suppressed by EME and MFME. The anti-arthritic properties of EME and MFME are a result of the combined effects of reducing lysosomal enzyme release, enhancing collagen and GAG synthesis, enhancing antioxidant system, and suppressing proinflammatory cytokines, which may have therapeutic benefits for the treatment of rheumatoid arthritis.The synergistic activity of several polyphenols, including flavonoids, tannins, and phenolic acids like chlorogenic acid and quercetin, may be responsible for Merremia emarginata's improved protective impact. The plant's positive effects are also a result of the presence of vital components like vitamin C, zinc, and copper. In lab tests, polyphenols have shown a restriction on cartilage deterioration and a maintenance of joint structure in rats with arthritis brought on by CFA. A vital nutrient found in plants, vitamin C, is required for connective tissue regeneration. It works as a particular inducer of the collagen biosynthesis pathway and makes it easier for proline to be converted to hydroxyproline, which is a crucial component of collagen. Merremia emarginata has a strong anti-arthritic effect, which is probably a result of its capacity to lessen pathological lesions by regulating proinflammatory cytokine levels. A decrease in acute phase proteins, which are indicators of inflammation, follows from this. Its promise as a successful treatment for rheumatoid arthritis is further supported by the plant's improved immunomodulatory characteristics. In conclusion, effective treatments for complicated diseases like arthritis may lie in a holistic strategy that targets a variety of mediators and their consequences. The results of this study, along with Merremia emarginata's traditional use, provide fresh opportunities for further investigation and the examination of its medicinal potential.

Conflict of Interest: No conflict of interest

Funding: This work was not financially support

Data availability: Data will be made available on request. Ethical considerations: The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), with the reference number IAEC/XXIX/10/2010

List of Abbreviations

ESR: erythrocyte sedimentation rate

RA: rheumatoid arthritis

ACP: acid phosphatase

CFA: complete freunds adjuvant

EME: ethonolic extract of *Merremia emarginata*

MFME: Methanolic extract of *Merremia emarginata*

TNF: tumor necrosis factor

CP: ceruloplasmin

ELIZA: enzyme-linked immunosorbent assay

HRP: antibodies IgG-horseradish peroxidase

OPD: o-phenylenediamine

SSB: sample solubilizing buffer

REFERENCE

1. David L Scott, Frederick Wolfe, Tom W J Huizinga., Rheumatoid arthritis (2010) Lancet 376: 1094–1108. doi: 10.1016/S0140-6736(10)60826-4.PMID: 20870100.
2. Cragg GM, Newman DJ, Snader KM (1997) Natural products in drug discovery and development. J Nat Prod 60: 52- 60. Doi: 10.1021/np9604893.
3. Mariana Laundry de Mesquita, Jose Elias de Paula, Claudia Pessoac, ManoelOdorico de Moraes, Leticia Veras Costa-Lotufo, Raphael Grougnet et al (2009) Cytotoxic activity of

Brazilian Cerrado plants used in traditional medicine against cancer cell lines. J. Ethn.pharm 23: 439–445. Doi: 10.1016/j.jep.2009.03.018.

4. Ekor M (2014) The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol 4:177. doi: 10.3389/fphar.2013.00177.
5. Bendele (2001) Animal models of rheumatoid arthritis,J Musculoskel Neuron Interact 1(4): 377-385. PMID: 15758488
6. Gary S. Firestein (2003) Evolving concepts of rheumatoid arthritis: Nature 423: 356-358. doi: 10.1038/nature01661.
7. Krishnaswamy Kannan, Robert A, Ortmann, Donald Kimpel(2005) Animal models of rheumatoid arthritis and their relevance to human disease. Pathophysiology 12: 167–181. Doi: 10.1016/j.pathophys.2005.07.011.
8. Newbould (1963) Chemotherapy of arthritis induced in rats by mycobacterial adjuvant. Brit. J. Pharmacol 21: 127-136. Doi: 10.1111/j.1476-5381.1963.tb01508.x.
9. Bina Joe, Ronald L Wiider. Animal models of rheumatoid arthritis. Molec Med. 1999; 5:367-369. doi: 10.1002/eji.200939578.
10. Yu Y, Xiong Z, Lv Y, Qian Y, Jiang S(2006) In vivo evaluation of early disease progression by X-ray phase-contrast imaging in the adjuvant-induced arthritic rat. Skeletal Radiology 35 (3): p.156–164. doi: 10.1007/s00256-005-0026-8.
11. Kalpesh Ramdas Patil, Chandragouda Raosaheb Patil, Ramchandra Baburao Jadhav,Vallabh Krishnalal Mahajan, Prabhakar Raosaheb Patil, and Pradeep Sampatrao Gaikwad. Anti-Arthritic Activity of Bartogenic Acid Isolated from Fruits of Barringtonia racemosa Roxb. (Lecythidaceae). Evidence-Based Complementary and Alternative Medicine Volume 2011;2011:785245. doi: 10.1093/ecam/nep148.
12. Otari KV, Shete R V, Upasani C D, Adak S S, Bagade M Y(2010) Evaluation of anti-inflammatory and anti-arthritic activities of ethanolic extract of *Vernonia anthelmintica* seeds. J of Cell and Tissue Resch 10(2): 2269-2280.
13. Shanmugapriya Ekambaram, Senthamil Selvan Perumal, Venkataraman Subramanian (2010) Evaluation of antiarthritic activity of *Strychnospotatorum* Linn seeds in Freund's adjuvant induced arthritic rat model. BMC Complementary and Alternative Medicine. doi: 10.1186/1472-6882-10-56.
14. Ekambaram Shanmugapriya, Subramanian Venkataraman (2006) Toxicological investigations on Strychno spotatorum Linn seeds in experimental animal models. J of health sci 52 (4): 339-343.doi: 10.1248/jhs.52.339.
15. J. King (1965) Practical Clinical Enzymology. (Ed. 2) The phosphohydrolases acid and alkaline phosphatases, in: D. Van Nostrand Company Limited 191–208. doi: 10.1007/BF00925707.
16. Delvin, Gianetto R (2009) The purification of lysosomal rat liver β- glucuronidase, Biochem. Biophys. Acta 220: 93–100. doi: 10.1016/0005-2744(70)90232-9.
17. Marhur N (1976) Rapid colorimetric assay of β-galactosidase and N-acetyl- β-d-glucosaminidase in human urine: Clin. Chim. Acta 73: 453–461.
18. Sapolsky AL, Altman RD, Howell, Cathepsin D (1973) activity in normal and osteoarthritic human cartilage: Fed. Proc. 32:1489–1493. doi: 10.1080/10496475.2011.632115.
19. Tettamanti G, Banali F (1983) A new procedure for the extraction, purification and fractionation of brain gangliosides: Biochim. Biophys. Acta 296 :160–170.

20. Rajendran Mythilypriya, Palanivelu Shanthi, PanchanadamSachdanandama (2008) Efficacy of Siddha formulation Kalpaamruthaa in ameliorating joint destruction in rheumatoid arthritis in rats. Chem.-Bio. Int 176 : 243–251. doi: 10.1016/j.cbi.2008.07.008.
21. Smith-Oliver, Stimpson S S, Yarnall D, Connolly K (1993) Elevated levels of TNF in the joints of adjuvant arthritic rats Cytokine 5: 298–304. doi: 10.1016/1043-4666(93)90060-i.
22. Sharma M, Singh (1989) Immunoassays Murine and Human Monoclonal Antibodies, Practical Manual National Institute of Immunology, New Delhi: 27–29.
23. Lowry OH, Rosenbrough NJ,Farn AL (1951)Protein measurement with Folin's phenol reagent. J. Biol. Chem 193 : 176–265.
24. Rajendran Mythilypriya, Palanivelu Shanthi, Panchanadam Sachdanandama (2008) Ameliorating effect of Kalpaamruthaa, a siddha preparation in adjuvant induced arthritis in rats with reference to changes in proinflammatory cytokines and acute phase proteins, Chem.-Bio. Int 179 :335–343.doi: 10.1016/j.cbi.2009.01.001.
25. Ekins S, Mestresand J (2007) In silico pharmacology for drug discovery: applications to targets and beyond, *Britis J of Pharmacol* 152 (1):21–37.doi: 10.1038/sj.bjp.0707306.
26. Mythilypriya R, Shanthi P, Sachdanandam P (2008) Efficacy of Siddha formulation Kalpaamruthaa in ameliorating joint destruction in rheumatoid arthritis in rats. Chem Biol Interact 176(2-3):243-51. doi: 10.1016/j.cbi.2008.07.008.
27. Lowry , Rosenbrough N, Farn AL (1951) Randall Protein measurement with Folin's phenol reagent. J. Biol. Chem 193 : 176–265.
28. Mythilypriya R, Shanthi P, Sachdanandam P(2008) Salubrious effect of Kalpaamruthaa, a modified indigenous preparation in adjuvant-induced arthritis in rats--a biochemical approach. Chem Biol Interact 28;173(2):148-58. doi: 10.1016/j.cbi.2008.02.007.
29. Mythilypriya R, Sachdanandam PS, Sachdanandam P(2009) Ameliorating effect of Kalpaamruthaa, a Siddha preparation in adjuvant induced arthritis in rats with reference to changes in proinflammatory cytokines and acute phase proteins. Chem Biol Interact 15;179(2-3):335-43. doi: 10.1016/j.cbi.2009.01.001. (35)
30. Elumalai EK, Ramachandran M, Thirumalai T, Vinothkumar P (2011) Antibacterial activity of various leaf extracts of *Merremia emarginata*. Asian Pac J Trop Biomed. 2011 Oct;1(5):406-8. doi: 10.1016/S2221-1691(11)60089-0.
31. Ekambaram S, Perumal SS, Subramanian V(2010) Evaluation of antiarthritic activity of Strychno spotatorum Linn seeds in Freund's adjuvant induced arthritic rat model. BMC Complement Altern Med.13;10:56. doi: 10.1186/1472-6882-10-56.
32. Purushoth Prabhu T , Panneerselvam P , Vijaykumar R , Clement Atlee W , Balasubramanian S(2012) Anti-inflammatory, anti arthritis and analgesic effect of ethanolic extract of whole plant of Merremia Emarginata Burm.F, Cent Eur J Exp Bio 1 (3):94-99 (http://scholarsresearchlibrary.com/archive.html).
33. Purushoth Prabhu.T, Shine Sudhev, Clement Atlee.W, R.Suresh, S.Balasubramanian, Pharmacognostic Studies on whole Plant of *Merremia emarginata* Burm. F (Convolvulaceae) , *International Journal of*

Pharmacognosy and Phytochemical Research 2014-15; 6(4); 1002-1006.

34. Yuan X., Han B., Feng Z.M., Jiang J.S., Yang Y.N., Zhang P.C. Chemical constituents of Ligusticum chuanxiong and their anti-inflammation and hepatoprotective activities. *Bioorg. Chem.* 2020;101:104016. doi: 10.1016/j.bioorg.2020.104016.
35. Zhang C., Fan L., Fan S., Wang J., Luo T., Tang Y., Chen Z., Yu L. Cinnamomum cassia Presl: A Review of Its Traditional Uses, Phytochemistry, Pharmacology and Toxicology. *Molecules.* 2019;24:3473. doi: 10.3390/molecules24193473.
36. Gai W., Hao X., Zhao J., Wang L., Liu J., Jiang H., Jin H., Liu G., Feng Y. Delivery of benzoylaconitine using biodegradable nanoparticles to suppress inflammation via regulating NF-κB signaling. *Colloids Surf. B Biointerfaces.* 2020;191:110980. doi: 10.1016/j.colsurfb.2020.110980.
37. Huang, P.; Hu, F.; Yang, Z.; Pan, Y.; Zhou, R.; Yan, Y.; Wang, H.; Wang, C. Matrine regulates Th1/Th2 inflammatory responses by inhibiting the Hsp90/NF-κB signaling axis to alleviate atopic dermatitis. *Kaohsiung J. Med Sci.* **2023**, *39*, 501–510.

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