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Evaluation of Anti-Arthritic Activity of Ethanolic Extract of *Murraya koenigii* Linn. Leaves in Complete Freund's Adjuvant Induced Rheumatoid Arthritis in Wistar Rats

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ABSTRACT

Rheumatoid arthritis (RA) is an inflammatory autoimmune disease that affects the joints causing joint deformity and physical disability. *Murraya koenigii* commonly known as curry leaf or karipatta in Indian dialects, belonging to Family Rutaceae. The curry leaf has several medicinal properties such as Anti-Diabetic, Antioxidant, Antimicrobial, Anti-Inflammatory, Anticarcinogenic, and Hepato-Protective Properties. Medicines derived from natural sources are known as phytomedicines which are clinically safe and effective due to a lesser number of side effects and effective therapeutic index. Thus, the demand for use of herbal medicines has been increased nowadays. The present work is to evaluate the effect of *Murraya koenigii* leaves for the management of rheumatoid arthritis and to give safe and efficient treatment by *In vivo* studies like measuring Paw edema volume, body weight, and *in vitro* study by protein denaturation.

INTRODUCTION:

Rheumatoid arthritis (RA) is a chronic, and systemic inflammatory autoimmune disease[1]. Rheumatoid arthritis is characterized by synovial inflammation and hyperplasia ("swelling"), autoantibody production (rheumatoid factor and anti-citrullinated protein antibody [ACPA]), cartilage, and bone destruction ("deformity"), and systemic features, including cardiovascular, pulmonary, psychological, and skeletaldisorders[2]. The disease, if untreated, often leads to permanent joint damage, significant impairment in quality of life, and eventual disability[1]. The immune system is well-organized and well-regulated. The deregulation of the immune system may lead to the development of autoimmune diseases[3]. Women are three times more susceptible than men and the disease is more frequent at the age of 40–50 years. Pro-inflammatory cytokines such as tumor necrosis factor –α (TNF –α), interleukin (IL)-1β, and IL-6 are important mediators of the disease perpetuation. Arthritis usually begins in the small joints of the hand and the feet, spreading later to the larger joints, the inflamed joint lining or synovial extends and then erodes the articular cartilage and bone, causing joint deformity and progressive physical disability.

The current pharmacologic therapies for the treatment of RA are NSAIDs, Glucocorticoids, DMARDs, other antirheumatic drugs, and Biological agents. *Murraya koenigii* commonly known as curry leaf or karipatta in Indian dialects, belonging to Family Rutaceae.

MATERIALS AND METHODS:

COLLECTION AND EXTRACTION OF PLANT MATERIAL

Fresh leaves of *Murraya koenigii* were collected/purchased from Chennai district, kodambakkam. It was identified and authenticated by Prof. P. Jayaraman, Ph.D., Director, Institute of herbal botany, Plant anatomy and research center, Chennai, Tamil Nadu, India. The extraction process is done by using a Soxhlet apparatus, which is a Hot extraction method.

IN-VIVO ANTI-ARTHRITIC STUDY

Induction of Arthritis

The animals were induced with a single injection of 0.1 ml of Complete Freund's Adjuvant which is dissolved in Liquid Paraffin in the concentration of 1mg/ml into the Sub-plantar region of the left hind paw.

Treatment Regimen:

1. **Group I** - Animals are given normal saline/p.o.

2. **Group II** - 0.1 ml of Complete Freund's Adjuvant induced Arthritis in Sub-Plantar region of the left hind paw.

3. **Group III** –Complete Freund's adjuvant-induced Arthritis animals treated with standard, 0.75 mg/kg Methotrexate/ p.o.

4. **Group IV** – Complete Freund's adjuvant-induced Arthritis animals treated with 200 mg/kg of EEMK /p.o.

5. **Group V** – Complete Freund's adjuvant-induced Arthritis animals treated with 400 mg/kg of EEMK /p.o.

Group II was induced with a single injection of 0.1ml Complete Freund's Adjuvant dissolved in liquid paraffin into the Sub-plantar region of the left hind paw on Day 0. Group III was treated with methotrexate (0.75mg/kg) as a standard drug after inducing arthritis with 0.1ml of Complete Freund's Adjuvant for 21 days. The fourth and fifth groups were treated with ethanolic extract of *M. Koenigii* (200mg/kg) and (400mg/kg) for 21 days.

PAW EDEMA VOLUME

Paw edema volume in each animal was measured on days 1,7,14, 21 is measured using Plethysmometer.

BODY WEIGHT:

The body weight was recorded on days 1, 7, 14, 21.

IN-VITRO ANTI-ARTHRITIC STUDY

PROTEIN DENATURATION

Procedure:

100µl of the test was added with 500µl of 1% BSA. The mixture was incubated for 10 minutes at 37ºC. Heat the contents in a water bath at 51ºC for 20 minutes. Cooldown to room temperature and check the absorbance at 660nm against the blank. Acetyl Salicylic acid was used as positive control and water as product control. Mizushima Y *et al*.

$$\% = 100-\{(A1-A2)/A0*100\}$$

X-RAY RADIOGRAPHY OF JOINTS: on day 22 Radiography of left knee joints were taken. The severity of the joint and bone deformation was scored according to the extent of osteoporosis, joint spaces, and joint structure on a scale of 0-4.

0- No degenerative joint changes.

1- Slightly soft tissue swelling, joint space narrowing, and degenerative joint changes.

2- Low to moderate soft tissue volume, joint space narrowing, and degenerative joint changes.

3- Pronounced soft tissue volume, joint space narrowing, and degenerative joint changes.

4- Excess soft tissue volume, joint space narrowing, and degenerative joint changes.

STATISTICAL ANALYSIS

Data were analyzed using one-way ANOVA followed by Dunnett's test and expressed as Mean± Standard Error of Mean (SEM). Statistical analyses were performed using Graph Pad Prism version 8.01, for windows. Differences between mean values of different groups were considered statistically significant at (P<0.0001)**** (P<0.001)***, (P<0.01)**, (P<0.05)*, ns- non significant.

RESULTS AND DISCUSSION:

RESULTS:

IN-VIVO ANTI-ARTHRITIC STUDY RESULTS

TABLE NO. 1: EFFECT OF EEMK ON CFA INDUCED RHEUMATOID ARTHRITIS RATS IN PAW EDEMA VOLUME OF LEFT HIND PAW

Paw edema volume on Complete Freund's Adjuvant induced RA model				
	1st DAY	**7th DAY**	**14th DAY**	**21st DAY**
GROUP I	0.45±0.07	0.50±0.05	0.50± 0.01	0.50 ± 0.01
GROUP II	0.52± 0.0 a*	0.62 ±0.02 a***	0.65± 0.01 a***	0.70 ± 0.01 a***
GROUP III	0.48± 0.04 $a^{ns}b^{ns}$	0.36 ± 0.02 a*** b***	0.32± 0.01 a*** b***	0.30 ± 0.03 a*** b***
GROUP IV	0.50 ± 0.01 $a^{ns}b^{ns}$ c^{ns}	0.40± 0.01 a** b*** c^{ns}	0.38 ± 0.00 a*** b*** c**	0.35 ±0.01 a** b*** c^{ns}
GROUP V	0.48 ± 0.01 $a^{ns}b^{ns}c^{ns}$	0.29 ± 0.01 a *** b*** c*	0.25 ± 0.01 a*** b*** c**	0.22 ± 0.04 a*** b*** c**

Values are expressed as Mean ±SEM of six animals

Group, I is compared with Group II, III, IV, and V is considered as 'a'.

Group II is compared with Group III, IV, and V is considered as 'b'.

Group III is compared with Group IV and V is considered as 'c'.

The Statistical significance test for comparison was done by One-way ANOVA followed by Dunnett's multiple comparisons tests where,**** is (P<0.0001),*** is (P<0.001), ** is (P<0.01),* is (P<0.05), ns is non-significant.

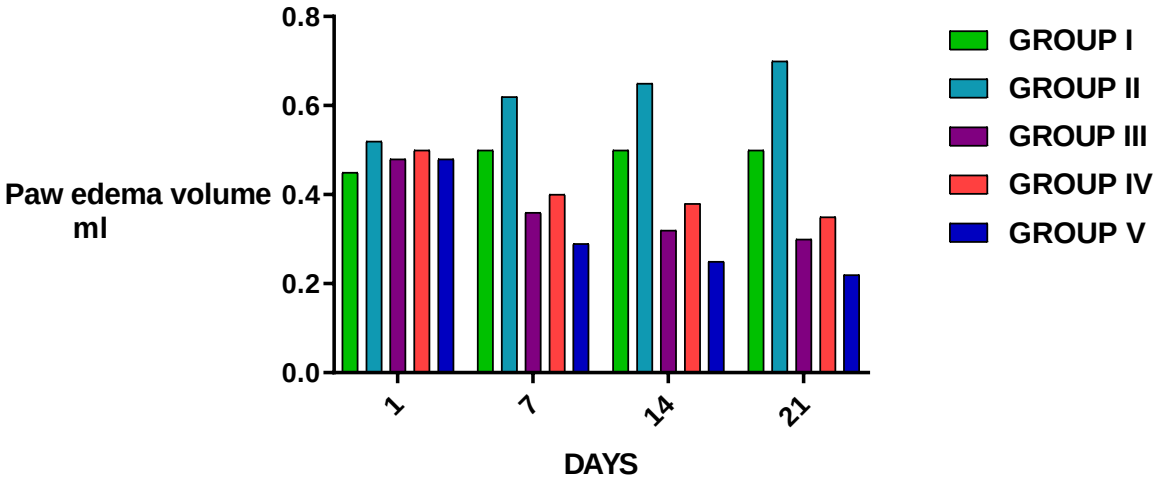


FIGURE NO. 1: EFFECT OF EEMK ON PAW EDEMA VOLUME CFA INDUCED RA RATS

TABLE NO. 2: EFFECT OF EEMK ON CFA INDUCED RHEUMATOID ARTHRITIS RATS IN BODY WEIGHT

Body Weight on Complete Freund's Adjuvant induced RA model				
	1st DAY	**7th DAY**	**14th DAY**	**21st DAY**
GROUP I	100±3.3	120±2.5	150±1.8	160±5.5
GROUP II	120±5.4 a**	110±8.4 a*	100±7.2 a***	80±3.5 a***
GROUP III	120±5.2 a**b^{ns}	140±5.4 a**b***	160±11.3 a*b***	180±13.4 a**b****
GROUP IV	110±4.1 a*b*c*	120±7.1 a^{ns}b*c**	135±6.8 a**b**c**	140±11.4 a**b****c***
GROUP V	125±8.3 a**$b^{ns}c^{ns}$	135±7.2 a**b**c^{ns}	180±12.1 a***b****c**	210±11.0 a***b****c**

Values are expressed as Mean ±SEM of six animals

Group, I is compared with Group II, III, IV, and V is considered as 'a'.

Group II is compared with Group III, IV, and V is considered as 'b'.

Group III is compared with Group IV and V is considered as 'c'.

The Statistical significance test for comparison was done by One-way ANOVA followed by Dunnett's multiple comparisons tests where,**** is (P<0.0001),*** is (P<0.001), ** is (P<0.01),* is (P<0.05), ns is non-significant.

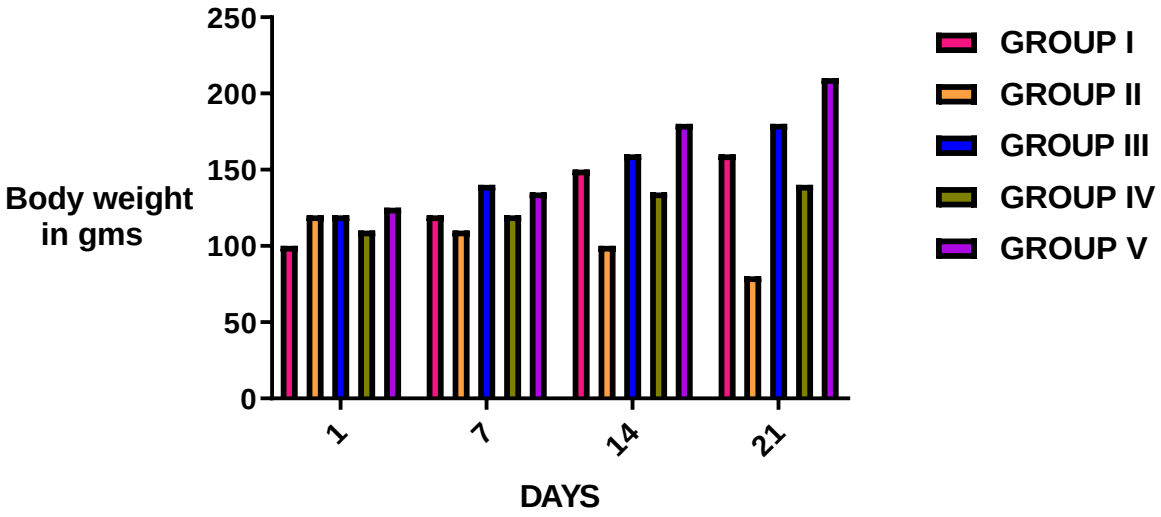


FIGURE NO. 2: EFFECT OF EEMK ON BODY WEIGHT CFA INDUCED RA RATS

***IN VITRO* ANTI-ARTHRITIC STUDY RESULTS**

TABLE NO. 3: EFFECT OF EEMK ON CFA INDUCED RHEUMATOID ARTHRITIS RATS IN PROTEIN DENATURATION

Protein denaturation on Complete Freund's Adjuvant induced RA model				
Concentration (µg/ml)	**20 µg/ml**	**40 µg/ml**	**60 µg/ml**	**80 µg/ml**
GROUP I	27.81	30.22	32.51	35.53
GROUP II	52.48 a****	54.11 a****	56.39 a****	58.64 a****
GROUP III	35.18 a**b**	38.13 a**b**	42.11 a**b**	46.99 a**b**
GROUP IV	40.9 a****b****	43.03 a****b****	45.86 a****b****	49.32 a****b****
GROUP V	51.12 a**b^{ns}	61.86 a**b^{ns}	70.48 a**b^{ns}	81.55 a**b^{ns}

Group, I is compared with Group II, III, IV, and V is considered as ‘a’.

Group II is compared with Group III, IV, and V is considered as ‘b’.

Group III is compared with Group IV and V is considered as ‘c’.

The Statistical significance test for comparison was done by One-way ANOVA followed by Dunnett’s multiple comparisons tests where, **** is (P<0.0001),*** is (P<0.001), ** is (P<0.01),* is (P<0.05), ns is non-significant.

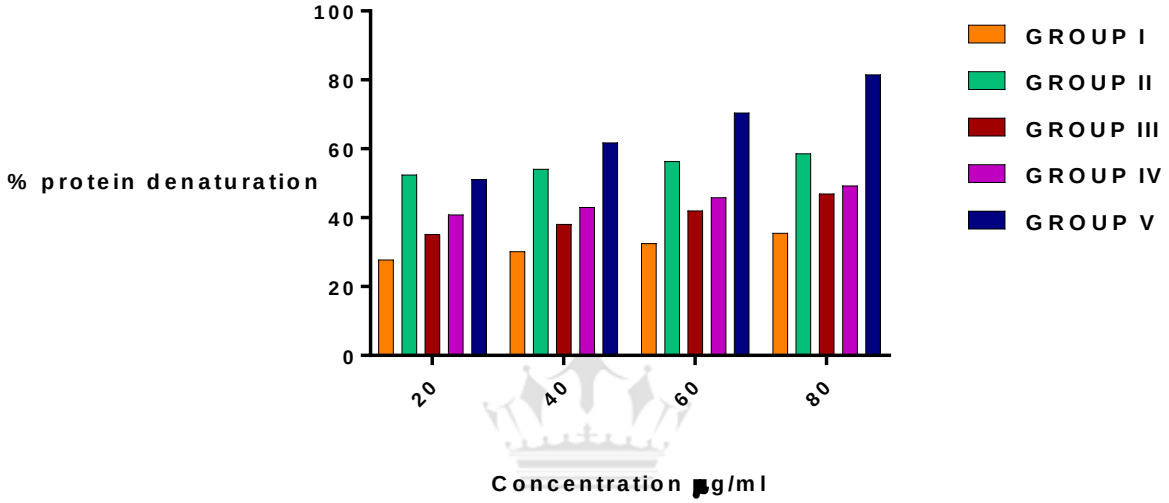
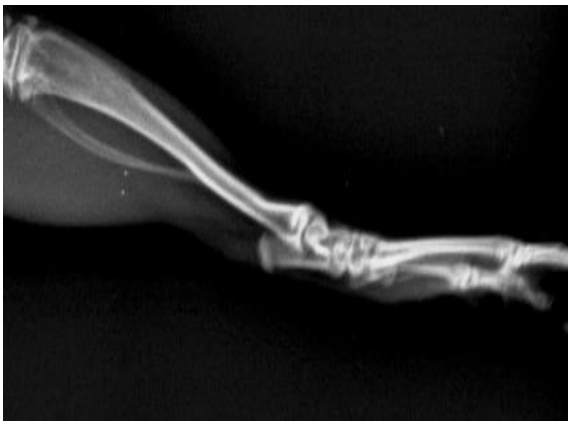
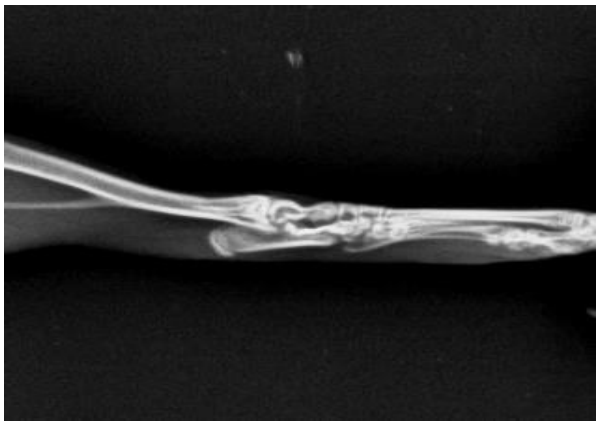


FIGURE NO. 3: EFFECT OF EEMK ON PROTEIN DENATURATION CFA INDUCED RA

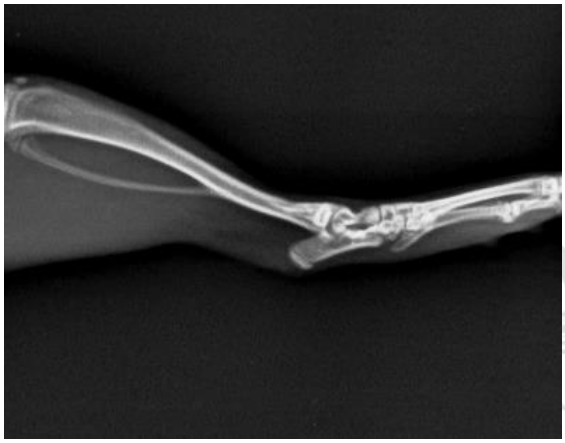
X-RAY RADIOGRAPHY ANALYSIS OF LEFT KNEE JOINTS OF RATS



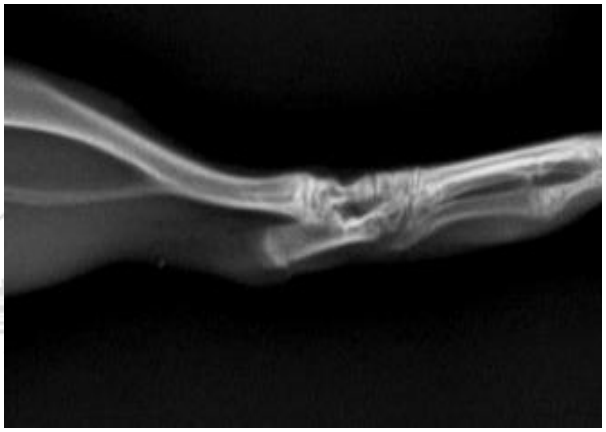
GROUP I



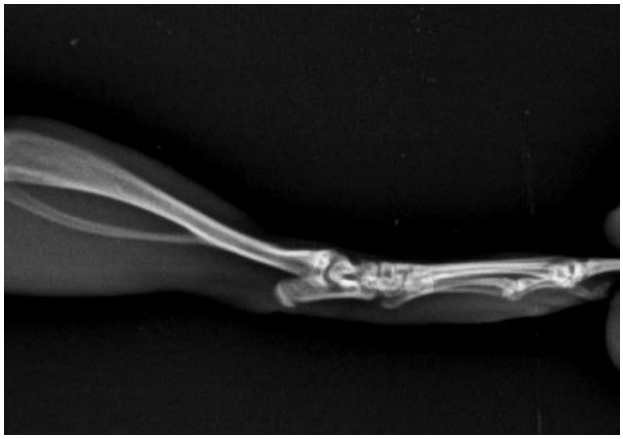
GROUP II



GROUP IV



GROUP III



GROUP V

FIGURE NO. 4: EFFECT OF EEMK ON CFA INDUCED RHEUMATOID ARTHRITIS RATS IN X-RAY RADIOGRAPHY ANALYSIS ON LEFT KNEE JOINTS

IN-VIVO ANTI-ARTHRITIC STUDY

Paw edema volume:

Effect of EEMK on CFA induced rheumatoid arthritic rats showing changes in paw edema volume

1st Day -Paw edema volume of Group II was significantly increased when compared with Group I (P<0.05). Paw edema volume of Group III, IV, V was non significantly increased when compared with Group I (ns). Paw edema volume of Group III, IV, V was non significantly increased when compared with Group II (ns). Paw edema volume of Group IV, V was non significantly increased when compared with Group III (ns). **(Table 1 & Figure 1)**

7th Day- Paw edema volume of Group II was significantly increased when compared with Group I (P<0.001). Paw edema volume of Group III (P<0.001), Group IV (P<0.01), Group V (P<0.001) was significantly decreased when compared with Group I. Paw edema volume of Group III, IV, V (P<0.001) was significantly decreased when compared with Group II. Paw edema volume of Group IV was non significantly decreased when compared with Group III and Group V (P<0.05) was significantly decreased when compared with Group III. **(Table 1 & Figure 1)**

14th Day- Paw edema volume of Group II was significantly decreased when compared with Group I (P<0.05). Paw edema volume of Group III, IV, V was significantly decreased when compared with Group I(P<0.001). Paw edema volume of Group III, IV, V was significantly decreased when compared with Group II (P<0.001). Paw edema volume of Group IV, V was significantly decreased when compared with Group III (P<0.01). **(Table 1 & Figure 1)**

21st Day- Paw edema volume of Group II (P<0.001) was significantly increased when compared with Group I. Paw edema volume of Group III (P<0.001). Group IV (P<0.01), Group V (P<0.001) was significantly decreased when compared with Group I. Paw edema volume of Group III, IV, V (P<0.001) was significantly decreased when compared with Group II. Paw edema volume of Group IV was non significantly decreased when compared with Group III and Group V (P<0.01) was significantly decreased when compared with Group III. **(Table 1 & Figure 1)**

Bodyweight:

Effect of EEMK on CFA induced rheumatoid arthritic rats showing changes in Bodyweight:

1st Day- Bodyweight of Group II (P<0.01), Group III (P<0.01), Group IV (P<0.05), and Group V (P<0.01) was significantly increased when compared with Group I. Body weight of Group III and Group V was non significantly increased when compared with Group II. The bodyweight of Group IV (P<0.05) was significantly increased when compared with Group II. The bodyweight of Group IV was significantly increased when compared with Group III (P<0.05). The bodyweight of Group V was non significantly increased when compared with Group III. **(Table 2 & Figure 2)**

7th Day- Bodyweight of Group II was significantly decreased when compared with Group I (P<0.05). The bodyweight of Group III was significantly increased when compared with Group I (P<0.01). The bodyweight of Group IV was nonsignificant when compared with Group I. Body weight of Group V was significantly increased when compared with Group I (P<0.001). Bodyweight of Group III (P<0.001)., Group IV (P<0.05), Group V (P<0.001) was significantly increased when compared with Group II. The bodyweight of Group IV (P<0.01) was significantly decreased when compared with Group III. Paw edema volume of Group V was not significantly decreased when compared with Group III. **(Table 2 & Figure 2)**

14th Day- Bodyweight of Group II was significantly decreased when compared with Group I (P<0.001). Paw edema volume of Group III (P<0.05), Group IV (P<0.01), Group V (P<0.001) was significantly increased when compared with Group I (P<0.05). Bodyweight of Group III (P<0.001), Group IV (P<0.01), Group V (P<0.001) was significantly increased when compared with Group II. The bodyweight of Group IV was significantly decreased when compared with Group III (P<0.01). The bodyweight of Group V was significantly increased when compared with Group III (P<0.01). **(Table 2 & Figure 2)**

21st Day- Bodyweight of Group II was significantly decreased when compared with Group I (P<0.001). Bodyweight of Group III (P<0.01), Group IV (P<0.01), Group V (P<0.001) was significantly increased when compared with Group I. Body weight of Group III, IV, V was significantly increased when compared with Group II (P<0.0001). The bodyweight of Group IV was significantly increased when compared with Group III (p<0.001). Bodyweight of

Group V was significantly increased when compared with Group III (P<0.01). (**Table 2 & Figure 2)**

***IN-VIVO* ANTI-ARTHRITIC STUDY**

Effect of EEMK on protein denaturation in CFA induced rheumatoid arthritic rats

When compared to Group I there was a significant increase in Protein denaturation in Group II (P<0.0001), Group III (P<0.01), Group IV (P<0.0001), and Group V (P<0.01). When compared to Group II there was a significant decrease in Protein denaturation in Group III (P<0.01) and Group IV (P<0.0001). When compared to Group II, there was no significant increase in Protein denaturation in Group V (ns). When compared to Group III, there was a significant decrease in Protein denaturation in Group IV (P<0.05) and Group V (P<0.01). **(Table 3 & Figure 3)**

X-RAY RADIOGRAPHY OF JOINTS

Group I-The X-ray radiographic of the knee joint of the rat shows no degenerative joint changes. **(Figure 4)**

Group II-The X-ray radiographic of the knee joint of rat shows excess soft tissue swelling, joint space narrowing, and degenerative joint changes. **(Figure 4)**

Group III-The X-ray radiographic of the knee joint of rat shows moderate soft tissue swelling, joint space narrowing, and degenerative joint changes. **(Figure 4)**

Group IV-The X-ray radiographic of the knee joint of rat shows pronounced soft tissue swelling, joint space narrowing, and degenerative joint changes. **(Figure 4)**

Group V-The X-ray radiographic of the knee joint of the rat shows low soft tissue swelling, joint space narrowing, and degenerative joint changes. **(Figure 4)**

DISCUSSION:

Rheumatoid arthritis (RA) is a chronic and systemic inflammatory autoimmune disease. It is characterized by synovial inflammation and hyperplasia. If left untreated, it often leads to permanent joint damage, significant impairment in quality of life, and eventual disability. The deregulation of the immune system may lead to the development of autoimmune diseases.

The prevalence of rheumatoid arthritis varies between 0.3% and 1% worldwide and is more in developed countries mainly affecting women than men (3:1)[1]. About 19,965,115 population are affected worldwide with rheumatoid arthritis, while 7 million are affected in India.

The inflammatory mediators are responsible for the development of clinical symptoms of inflammation. They cause vasodilation, increased permeability of blood vessels, and migration of leukocytes to the site of inflammation. Cytokines are locally acting protein mediators that are involved in almost all the biological processes including cell growth and activation, inflammation, immunity, and differentiation. the expression of cytokines at mRNA levels in patients with arthritis has revealed that many pro-inflammatory cytokines are abundant in synovial tissues. The increased expression of inflammatory mediators in arthritic joints is counteracted to some degree by the production of anti-inflammatory Th2 cytokines. Since the balance of Th1/Th2 cytokines is thought to influence autoimmune diseases like arthritis.[8]

Although the causes of RA remain unknown, some studies have revealed that cytokines play an important role in the inflammation cascade and some of the important pro-inflammatory cytokines involved in RA such as interleukin 1 (IL-1) and tumor necrosis factor (TNF-a) are thought to play key roles in the destruction of cartilaginous and bony tissues in joints affected by RA. These inflammatory cells secrete lysosomal enzymes which damage cartilage and erode bone, while PGs produced in the process cause vasodilation and pain.[8] There are two types of Rheumatoid Arthritis: Seropositive Rheumatoid Arthritis and Seronegative Rheumatoid Arthritis. Seropositive RA shows an increased risk for bone erosion and joint damage. Seronegative RA patients are often present with higher inflammatory activity.

The management of rheumatoid arthritis is a multidisciplinary approach to lessen the pain, reduction of inflammation, and restorative joints function. In practical terms suppression of inflammation is the target intensive therapy. Beneficial effects in RA are probably related to inhibition of cytokine production, chemotaxis, and cell-mediated immune reaction. Multiple known risk factors for RA are hypothesized to be related to the development of the immune response against citrullinated proteins and thus Anti-Citrullinated Protein Antibody formation.[2]

Herbal plants provide most of the medicinal needs. Important herbal products include spices, herbal teas, functional foods, medicinal raw materials, essential oils, flavoring, and dietary supplements. The medicinal use of the plant is a result of the phytoconstituents present in them. Some of these chemicals are bioactive and produce definite physiological and biochemical actions. *Murraya koenigii* is widely used in Indian cookery for centuries and has a versatile role to play in traditional medicine. This plant has been reported to have anti-oxidative, cytotoxic, antimicrobial, antibacterial, anti-ulcer, positive inotropic, and cholesterol-reducing activities. In the traditional system of Medicine, *Murraya koenigii* is used as antiemetic, antidiarrhoeal, dysentery, febrifuge, blood purifier, tonic, stomachic. The oil is used externally for bruises, eruptions, in the soap and perfume industry.[14]

Freund's adjuvant is used to induce arthritis widely which is a chronic model for inflammation like Rheumatoid Arthritis. One of the reasons for the wide utilization of this model is due to the strong correlation between the efficacy of therapeutic agents in this model and rheumatoid arthritis in humans and it is characterized by very rapid erosive disease. Persistent inflammation produces swollen joints with severe synovitis, decreased nociceptive threshold, and massive subsynovial infiltration of mononuclear cells, which along with angiogenesis leads to pannus formation. Expansion of the pannus induces bone erosion and cartilage thinning, leading to the loss of joint function. Freund's adjuvants traditionally had the following three specific mechanisms of action: 1-establishing an antigen depot with slow antigen release, 2-providing a vehicle for antigen transport throughout the lymphatic system to immune effector cells, and 3- interacting with antigen-presenting cells including phagocytes, macrophages, and dendritic cells. [16]

Methotrexate (DMARDs) is the appropriate first-line drug for most patients with RA. It is more effective, better tolerated, and less likely to cause adverse events than other disease-modifying antirheumatic drugs (DMARDs). About half of all patients have little or no radiographic progression, although 20% to 30% require biological DMARDs to control radiographic progression. A dose of 7.5 mg or 15 mg per week of methotrexate is given for the treatment of Rheumatoid arthritis. Adverse effects of Methotrexate are nausea, feeling tired, fever, increased risk of infection, low white blood cell counts, and breakdown of the skin inside the mouth, and birth defects in pregnant women. Methotrexate can harm the function of the liver. Mechanism of action of Methotrexate involves inhibition of enzymes involved in purine metabolism, leading to accumulation of adenosine; inhibition of T

cell activation and suppression of intercellular adhesion molecule expression by T cells; selective down-regulation of B cells; increasing CD95 sensitivity of activated T cells; and inhibition of methyltransferase activity, leading to deactivation of enzyme activity relevant to immune system function. Another mechanism of MTX is the inhibition of the binding of interleukin 1-beta to its cell surface receptor.[32]

Biological DMARDs are drugs that delay the progression of RA by affecting the body's biological response to various cytokines, particularly TNF-a. TNF-a is the primary cytokine responsible for the systemic inflammation iconic in RA and has been found at increased levels in patients with this pathology.[32]

Drugs used in the treatment of rheumatoid arthritis are commonly used DMARDs-Methotrexate, Hydroxychloroquine, Sulfasalazine, Leflunomide, Azathioprine. Unusual or rarely used DMARDs-Cyclosporine, Gold thiomalate aurothioglucose, oral auranofin, Minocycline, Penicillamine, Cyclophosphamide. Biological anti-TNF agents are Infliximab, Etanercept (Enbrel), Adalimumab, Golimumab, Certolizumab pegol. Other biological DMARDs are T cell co-stimulatory blocker – abatacept, Anti-CD20 - Rituximab, Anti-IL-6 receptor-Tocilizumab, Anti-interleukin-1 receptor blocker-Anakinra. NSAIDs like Ibuprofen, Naproxen, Diclofenac, Indomethacin, Nabumetone, Celecoxib.

The DMARDs Hydroxychloroquine may damage the eye's retina. Sulfasalazine causes sweat, tears, or urine to look orange and skin more sensitive to sunlight. There are rare reports that biologic DMARDs can increase the risk for some cancers, psoriasis, multiple sclerosis, and heart failure.

A decrease in body weight occurs in many chronic diseases, such as diabetes, cancer, as well as in inflammatory diseases such as Crohn's disease, sepsis, and RA. Therefore, body weight is used to assess the progression of the disease. [30]

The synovitis, swelling, and joint damage that characterize RA are the results of complex autoimmune and inflammatory processes that involve components of both the innate and adaptive immune systems. Collagen is gradually destroyed, narrowing the joint space and finally damaging bone that leads to paw edema swelling. [29]

Protein denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a

concentrated inorganic salt, an organic solvent, or heat. Most biological proteins lose their biological function when denatured. For example, enzymes lose their activity, because the substrates can no longer bind to the active site. Denaturation of protein is one of the causes of rheumatoid arthritis[27]. Production of autoantigen leads to denaturation of protein in certain arthritic diseases. Modulation of electrostatic, hydrogen, hydrophobic, and disulfide bonding in denaturation of protein, which is the mechanism of protein denaturation.[31]

Radiographic Analysis in RA is used in diagnosis which indicates the severity of the disease. Soft tissue swelling is the early sign, whereas later the prominent radiographic changes like bony erosions and narrowing of joint spaces are observed only in the final stages of arthritis. In the negative control, soft tissue swelling was severe along with narrowing of the joint spaces which is the cause for bone destruction in arthritic condition. Standard drug Methotrexate has prevented this bone destruction with moderate swelling and narrowing of the joint was observed.

Paw edema volume which is increased by Freund's Adjuvant dissolved in Liquid paraffin is decreased by EEMK.

The arthritis control has shown a decrease in body weight, while EEMK has shown a significant increase in Bodyweight when compared to arthritis control.

Protein denaturation has been decreased by EEMK when compared to arthritis control.

EEMK has shown significant prevention against bone destruction by showing less soft tissue swelling and narrowing of joint spaces in 400mg/kg dose in the 21 days of treatment when compared with negative control.

CONCLUSION:

Thus, it may be concluded that the Ethanolic extract of *Murraya koenigii* leaves has significant Anti-arthritic activity by decreasing the Paw edema volume, Protein denaturation, and increasing the Bodyweight in Complete Freund's adjuvant-induced Rheumatoid Arthritis model in rats.

The Anti-Rheumatic activity and Anti-inflammatory activity were found significant in *Murraya koenigii* leaves due to the presence of Monoterpenes like carvacrol, P-cymene, Alpha & Beta–Pinene.

Further studies to isolate the chemical constituents responsible for the Anti-Rheumatic activity and Anti-inflammatory activity is required.

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