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Research Article

EFFECT OF ETHANOLIC AND ETHYLACETATE EXTRACT OF *MERREMIA EMARGINATA* (BURM.F) IN RHEUMATOID ARTHRITIS

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ABSTRACTS

The effect of alcoholic and ethyl acetate extracts of the whole plant of *Merremia emarginata* (Burm. F) Hallier f. on freund's adjuvant induced arthritis was evaluated. The percentage of yield was found to be 18.7 and 10.6% for alcoholic and ethyl acetate extracts respectively. Both the extracts significantly ($p < 0.05$) decrease the paw thickness at the end of 21 days treatment. Though in acute phase inflammation both of them show the same potency in chronic phase alcoholic extract exhibit more potency than the ethyl acetate extracts. At the end of the studies the alcoholic extract shows more pronounce effect as comparable to ethyl acetate extract. The phytochemical analysis reveals the presence of flavonoids, glycosides, phenols, carbohydrates and proteins in the extracts. This result support the folkore use of this plant against the inflammmatory conditions like arthritis.

KEYWORDS: *Merremia emarginata*, adjuvant induced arthritis, whole plant, acute and chronic phase inflammation.

INTRODUCTION

Rheumatoid arthritis is an autoimmune disease characterized by chronic inflammation, hyperproliferation of the synovial lining and cartilage destruction. Cytokines, in particular tumor necrosis factor (TNF), are elevated in the synovial fluid and presumably involved in the disease process by upregulation of a multitude of inflammatory mediators[1,2]. This disease has a world wide distribution but its pathogenesis is not clearly understood[3] although there are few anti-rheumatic drugs showing effectiveness on the treatment of rheumatoid arthritis, the side effect and toxicity call for new and more effective natural drugs[4]. Rat adjuvant arthritis is an experimental model of polyarthritis which has been widely used for preclinical and clinical investigations. The pattern of inflammation by freund's adjuvant is similar pattern as arthritis in mammals[5]. *Merremia emarginata Burm*. F (Convolvulaceae) is a perennial, much branched herb (creeper). It is found widely distributed all over the India, specially in damp places in upper gangetic plain, Gujarat, Bihar, West Bengal, Western- Ghats, ascending up to 900m in the hills, Goa, Karnataka in India, Ceylon and Tropical Africa. *Merremia emarginata* is also known as *Ipomoea reniformis* chois[6]. It is adulterated with *Centella asiatica*[7]. It is reported to have many important medicinal properties. In the Indigenous system of Medicine, *Ipomoea reniformis* has been claimed to be useful for cough, headache, neuralgia, rheumatism, diuretic, inflammation, troubles of nose, fever due to enlargement of liver and also in kidney diseases. Powder of leaves is used as a snuff during epileptic seizures, Juice acts as purgative and the root is having diuretic, laxative, and applied in the disease of the eyes and gums[8]. The plant is reported to contain resin, glycosides, reducing sugars and starch. Petroleum ether extract was reported to contain fats and fixed oil while aqueous extract was reported to contain amino acids, and starch[9]. Chemical investigation of *Ipomoea reniformis* shows the presence of caffeic, p-coumaric, ferulic and sinapic acid esters identified in seeds[10].

MATERIALS & METHODS

Preparation of ethanol and ethyl acetate extract

The whole plant *Merremia emarginata* Burm. F were collected from Tirunelveli, Tamilnadu, India during Nov 2010 and was authenticated by Prof Jayaraman, PARC, Tambaram, Chennai. The fresh Plant material were washed, shade dried and pulverized in a mechanical grinder to obtain coarse powder. The coarse powder was extract with alcohol and ethyl acetate in a soxhlet extractor for 18 hours. The solvent was completely removed by distillation and dried in a vacuum desiccator. The total alcoholic and ethyl acetate extract obtained was screened for Antiarthritic activity[11].

Experimental animals

Colonies inbreed strains of Wistar albino rats weighing (200-250gm) of both sex were obtained from C. L. Baid Metha College of pharmacy was used for the pharmacological studies. The animals were kept under standard conditions maintained at 23-25°C, 12 hr light/dark cycle and given standard pellet diet (Hindustan lever, Bangalore) provided *ad libitum*. The animals were acclimatized to the laboratory conditions for a week prior to the experimentation and randomly divided into six groups of each six animals. Principles of animal handling were strictly adhered to the guidelines and handling of animals was made under the supervision of animal ethics committee of the institute. The experimental protocol was approved by Institutional animal ethics committee (IAEC) of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals[12]. **IAEC Reference number:** (IAEC/XXIX/10/2010).

Acute toxicity studies

This was performed for the extracts to ascertain safe dose by the acute oral toxic class method by the Organization of Economic Cooperation and Development (OECD). A single administration of starting dose of 2000 mg/kg body weight/po of the EME and EAME was administered to two male and one female rat, and the rat were observed for three days to evaluate considerable changes in body weight and other signs of toxicity. Repeating the experiment with the

same dose level of EME and EAME for more seven days, we observed the body weight change and toxicity sign for totally fourteen days[13].

ANTI ARTHRITIC ACTIVITY

Induction of arthritis

Arthritis was induced by a single sub-planter injection of 0.1 ml of Complete Freund's adjuvant (CFA)(Sigma Chemicals, USA) containing 1.0 mg dry heat-killed *Mycobacterium tuberculoi* per milliliter sterile paraffin oil into a foot pad of the left hind paw of rats . The swelling in hind paws were periodically examined in each paw from the ankle using digital Plethysmometer (Panlabs, India). Albino wistar rats of both sex used for the study[14].

Animals were randomly divided into seven groups of six animals each (n = 6). Group I served as control received 0.1ml of vehicle in which extract is going to be suspended group II served as negative control received 0.1ml freunds adjuvant, and Group III & IV received Ethanolic extract of *Merremia emarginata* (EME) at a dose of 200 mg/kg and 400 mg/kg. Group V & VI received Ethylacetate extract of *Merremia emarginata* (EAME) at a dose of 200mg/kg and 400mg/kg. Group VII received Methotrexate (10 mg/kg p.o) served as reference standard. Arthritis was induced by injecting 0.1 ml of freund's adjuvant into the left hind paw. Drug treatment was started from the initial day, that is, from the day of adjuvant injection (0 day), 30 min before adjuvant injection and continued till 21st day. Paw volume and Paw thickness was measured on 0, 4th, 8th, 14th and 21stdays by using Plethysmometer and verneir caliper respectively. The mean changes in injected paw edema with respect to initial paw volume, were calculated on respective days[15].The body weight of the animals were measured by digital balance to access the course of the disease at the initial day before induction and at the end of 21st day. The rats were anaesthetized under light ether anesthesia and blood was collected by retroorbital puncture for estimation of serum parameter such as Hb, RBC, WBC, ESR and CRP by using various diagnostic kits[16].

Paw volume measurement

On 0th day, the left hind paw volume of all rats as a volume displacement was measured using digital Plethysmometer and on 1st day arthritis was induced in all rats using CFA. The aforementioned drug treatment was started on 1st day and continued for 28 days. The assessment of anti-arthritic activity was carried out by measuring change in paw volume edema on 4th, 8th, 14thand 21st day after induction. The percent inhibition of paw volume of treated rats against vehicle treated rats was evaluated.

Paw diameter measurement

On 0th day, the left hind paw volume of all rats as a Diameter displacement was measured using Vernier Caliper and on 1st day arthritis was induced in all rats using CFA. The aforementioned drug treatment was started on 1st day and continued for 21 days. The assessment of anti-arthritic activity was carried out by measuring change in paw Diameter on 4th, 8th, 14th, 21stday after induction. The percent inhibition of Diameter of treated rats against vehicle treated rats was evaluated.

Biochemical Assays

After collection of blood it was added to sodium citrate to prevent from coagulation and the sample was centrifuged and serum was collected and given for the following blood analysis Haemoglobin content was estimated Red blood cell (RBC) and white blood cell (WBC) counts were estimated according to the method of in an improved Neubauer chamber. Estimation of erythrocyte sedimentation rate (ESR) was carried out by the C Reactive protein levels were estimated using the ELISA.

Statistical Analysis

Results were expressed as mean ± SD. The significance of difference among the groups was assessed using One way analysis of variance (ANOVA) followed by Dunnet's test. $P<0.05$ was considered significant.

HISTOLOGY

Rats were killed by ether anesthesia. Knee joints were removed and fixed for 4 days in 4 % formaldehyde. After decalcification in 5 % formic acid, the specimens were processed for paraffin embedding tissue sections (7 µm thick) and were stained with haematoxylin and eosin, or safranin. An experienced pathologist, unaware of the different drug treatments scored the condition of tibiotarsal joints.

Histopathological changes were scored using the following parameters. Infiltration of cells was scored on a scale from 0 to 3, depending on the amount of inflammatory cells in the synovial tissues. Inflammatory cells in the joint cavity were graded on a scale from 0 to 3 and expressed as exudate. A characteristic parameter in Freund's complete adjuant is the progressive loss of articular cartilage. This destruction was separately graded on a scale from 0 to 3, ranging from the appearance of dead chondrocytes (empty lacunae) to complete loss of the articular cartilage. Bone erosion was scored on a scale ranging from 0 to 3, ranging from no abnormalities to complete loss of cortical and trabecular bone of the femoral head. Cartilage and bone destruction by pannus formation was scored ranging from 0, no change; 1, mild change (pannus invasion within cartilage); 2, moderate change (pannus invasion into cartilage/subchondral bone); 3, severe change (pannus invasion into the subchondral bone); and vascularity (0, almost no blood vessels; 1, a few blood vessels; 2, some blood vessels; 3, many blood vessels). Histopathological changes in the knee joints were scored in the femur region on 5 semiserial sections of the joint, spaced 70 µm apart. Scoring was performed on decoded slides by two observers, as described earlier[17].

Radiography

Both sex wistar rats were sacrificed on 21st day of Freund's complete adjuvant administration and legs are removed and placed on formalin containing plastic bag. This plastic bag was kept at a distance of 90 cm from the X-ray source was and Radiographic analysis of arthritic and treated animal hind paw were performed by X-ray machine (International journal Electron Company) with a 300-mA exposition for 0.01 s. an investigator blinded for the treatment regime performed radiograph score. The following radiograph criteria were considered: These scores (destroyed or intact joint) were used as a quantal test for bone necrosis[18]. Radiographs were carefully examined using a stereo microscope and abnormalities were graded as follows:

- Periosteaic reaction, 0 - 3 (none, slight, moderate, marked);
- Erosions, 0 - 3 (none, few, many small, many large);
- Joint space narrowing, 0 - 3 (none, minimal, moderate, marked);
- Joint space destruction, 0 - 3 (none, minimal, extensive, ankylosis).

RESULTS AND DISCUSSIONS

Table: 1 PAW VOLUME CHANGES

Treatment	Day 1	Day 4	Day 8	Day 14	Day 21
Control	0.82 ± 0.6	0.82 ± 0.6	0.82 ± 0.6	0.82 ± 0.6	0.82 ± 0.6
Negative Control	0.94 ± 0.07	1.85 ± 0.16	2.42 ± 0.10	2.78 ± 0.13	2.67 ± 0.12
EME 200mg/kg p.o	0.90 ± 0.07	1.41 ± 0.07	1.93 ± 0.12	1.95 ± 0.17**	2.02 ± 0.13**
EME 400mg/kg p.o	0.92 ± 0.06	1.39 ± 0.11	1.87 ± 0.09	2.12 ± 0.19**	1.96 ± 0.16**
EAME 200mg/kg p.o	0.87 ± 0.2	1.67 ± 0.2	2.02 ± 0.09	2.24 ± 0.21	2.02 ± 0.15
EAME 400mg/kg p.o	0.93 ± 0.08	1.75 ± 0.10	2.10 ± 0.07	2.01 ± 0.6	1.96 ± 0.6*
Standard Drug	0.93 ± 0.06	1.28 ± 0.10*	1.41 ± 0.11**	1.44 ± 0.11***	1.35 ± 0.11**

The values are Means ± S.E.M. (n=6), * $P < 0.05$, ** $P < 0.01$, compared with standard drug treated group (one-way ANOVA followed by Dunnett's test)

Table: 2 PAW DIAMETER

Treatment	Day 1	Day 4	Day 8	Day 14	Day 21
Control	8.00 ± 0.11	8.00 ± 0.11	8.00 ± 0.11	8.00 ± 0.11	8.00 ± 0.11
Negative Control	9.00 ± 0.23	16.00 ± 0.47	21.00 ± 0.52	24.40 ± 0.67	22.00 ± 0.46
EME 200mg/kg p.o	9.00± 0.15	15.47 ± 0.55	18.67 ± 0.74	17.46 ± 0.09**	16.23 ± 0.36**
EME 400mg/kg p.o	8.43 ± 0.20	15.09 ± 0.13	19.23 ± 0.56	17.85 ± 0.10**	16.23 ± 0.11**
EAME 200mg/kg p.o	9.67 ± 0.23	15.75 ± 0.67	18.75 ± 0.67	18.72 ± 0.24	18.28 ± 0.5
EAME 400mg/kg p.o	8.47 ± 0.55	16.23 ± 0.74	15.91 ± 0.55	17.83 ± 0.56	16.87 ± 0.5**
Standard Drug	7.32 ± 0.13	12.00 ± 0.37	13.42 ± 0.12**	13.02 ± 0.24***	12.00 ± 0.22**

The values are Means ± S.E.M. (n=6), * $P < 0.05$, ** $P < 0.01$, compared with standard drug treated group (one-way ANOVA followed by Dunnett's test)

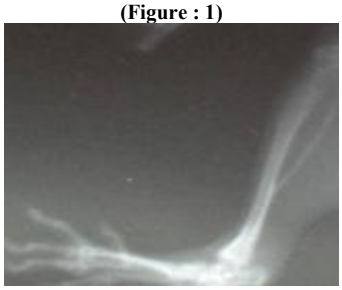
Table: 3 BLOOD PARAMETERS

Treatment	Hb Count (g/dl)	RBC Count ($x10^6$/ mm^3)	WBC Count ($x10^6$/mm^3)	ESR	CRP
Control	17.33 ± 0.76	5.2 ± 0.1	7.85 ± 0.8	6.17 ± 1.35	172.9 ± 1.5
Negative Control	9.7 ± 0.8	4.5 ± 0.1	8.37 ± 0.8	10.73 ± 0.4***	425.3 ± 1.6
EME 200mg/kg p.o	11.6 ± 0.92**	5.58 ± 0.1	7.13 ± 0.8	8.25 ± 0.3	413.1 ± 1.1
EME 400mg/kg p.o	15.7 ± 0.65**	5.65 ± 0.1	7.05 ± 0.8	6.37 ± 0.2***	228 ± 0.8**
EAME 200mg/kg p.o	10.3 ± 0.73	5.14 ± 0.1	7.11 ± 0.8	7.67 ± 0.4	385 ± 0.7
EAME 400mg/kg p.o	12.3 ± 0.67	5.23 ± 0.1	7.08 ± 0.8	5.98 ± 0.3	202 ± 0.73**
Standard Drug	18.6 ± 0.92	7.22 ± 0.1	7.15 ± 0.8	6.73 ± 0.2	263 ± 0.67

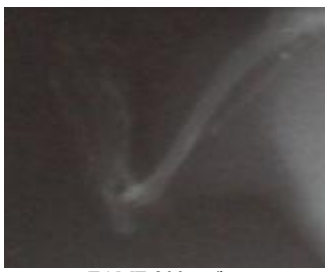
The values are Means ± S.E.M. (n=6), * $P < 0.05$, ** $P < 0.01$, compared with standard drug treated group (one-way ANOVA followed by Dunnett's test)

RADIOGRAPHIC PHOTO

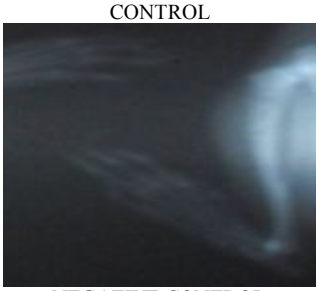
(Figure : 1)



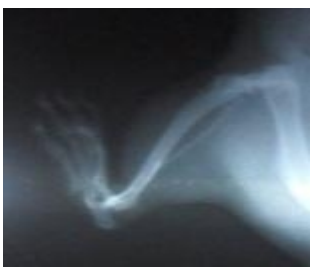
CONTROL



EAME 200mg/kg

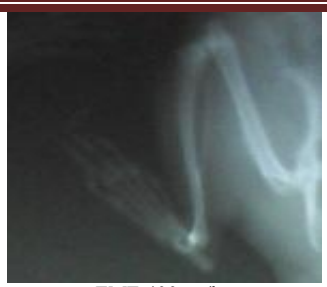


NEGATIVE CONTROL



EAME 400mg/kg

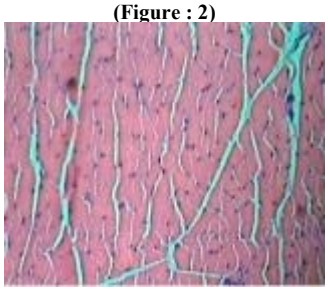
EME 200mg/kg



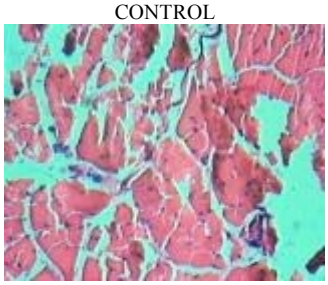
EME 400mg/kg

HISTOPATHOLOGY PHOTOS

(Figure : 2)

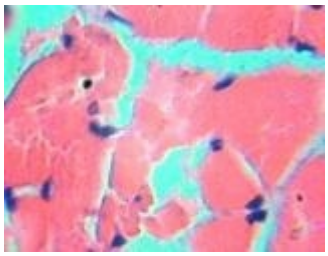


CONTROL

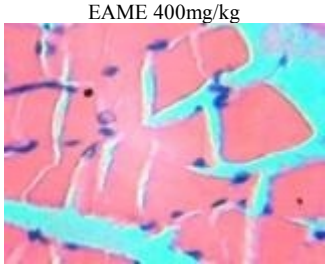


NEGATIVE CONTROL

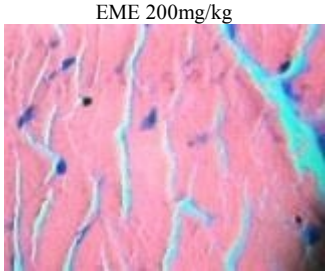
EAME 200mg/kg



EAME 400mg/kg



EME 200mg/kg



EME 400mg/kg

Rheumatoid Arthritis is an autoimmune disorder, the immunologically mediated complete Freund's adjuvant induced arthritic model of chronic inflammation is considered as the best available experimental model of RA[19].Complete Freund's adjuvant-induced arthritis is a model of chronic polyarthritis with features that resemble RA[20]. Evaluation of the inflammatory stratus in RA is reflected inflammation in the hind paw. The hind paw injected with complete Freund's adjuvant became gradually swollen and reached its peak at 21st day.

Table 1 showed the results obtained for the ethanolic, ethylacetate extract and the standard drug in the complete freund's adjuant-induced(CFA) paw volume test at specific time intervals. It was obvious that during 21st day treatment paw volume in disease control inflamed paw is increase in time dependent manner and 400mg/kg of EME treated group significantly inhibited the development of joint swelling induced by complete Freund's adjuant. Table 2 showed a significant thickness reduction in the paw thickness treated with dose 400mg/kg in the complete Freunds adjuvant induced arthritis in rats when compared to the standard drug Methotrexate treated animals. In the complete Freund adjuvant induced arthritis the dose at 400mg/kg of EME treated animals shows a significant reduction in Hb, RBC, WBC, ESR, and C-Reactive protein when compared to the standard drug Methotrexate treated animals(Table 3)[21]. Bone destruction, which is a common feature of adjuvant arthritis, was examined by radiological analysis (Figure:1). Adjuvant treated rats had developed definite joint space narrowing of the intertarsal joints, diffuse soft tissue swelling that included the digits, diffuse demineralization of bone, marked periosteal thickening, and cystic enlargement of bone and extensive erosions produced narrowing or pseudowidening of all joint spaces. Despite a similar clinical course of arthritis, disease control rats suffered from more pronounced bone destruction than ethanolic extract treated group. The animals treated with EME 400mg/kg group decreased cystic enlargement of bone and extensive erosion produced narrowing of all joint space, soft tissue swelling when compared to the standard drug Methotrexate treated animals[22]. Histopathological studies(Figure: 2) showed that compare to the normal control the Freund complete adjuvant induced arthritic control group showed the following changes in Haemotoxylin and eosin stained slide, Infilteration of synovial membrane, Accumulation of inflammatory cells, Replacement of articular cartilage by inflammatory cells. Histopathology studies of synovial joint showed that the treatment with EME 400mg/kg group decreased vascularity

lymphocytic infilteration with less rheumatoid inflammation and angiogenisis, with no thickening of synovial membrane and absence of lymphoid follicles as compared to the standard drug Methotrexate (5mg/kg) treated animals[23].

From the results observed from the current investigation, it is concluded that the alcoholic extract of *Merremia emarginata* Burm.F possesses potentially useful anti arthritic activity since it give a positive result in controlling inflammation in adjuvant induced arthritic model in rats.

CONCLUSION

The plant described in this paper, *Merremia emarginata*, is well documented in traditional Ayurveda system of medicine for the treatment of arthritis. The plant ethanolic extract possesses significant anti arthritic activity gives an important lead for detailed studies that can exploit its active ingredients.

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