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## Anti-inflammatory, anti arthritis and analgesic effect of ethanolic extract of whole plant of *Merremia Emarginata* Burm.F

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### ABSTRACT

To evaluate the anti-inflammatory, anti arthritis and analgesic activity of ethanolic extract of whole plant of *Merremia emarginata* in experimental animal models. Ethanolic extract of whole plant of (EME)(100, 200 and 400 mg/kg p.o) was studied for its anti inflammatory activity using carrageenan induced rat paw edema animal model, anti arthritis activity using complete freund's adjuvant model and analgesic activity at the same dose level using Hot plate analgesia in mice. The Percentage inhibition with indomethacin and EME in the carrageenan induced paw edema at the dose level 400mg/kg were 82% and 74% at the end of 5hr. The results indicate that treatment of adjuvant induced arthritic rats with EME improves ESR, Hb value and also restores body weight. Significant ( $P < 0.05$ ) inhibitory effect was observed with EME on FCA induced paw edema throughout the study. In radiant tail flick method the extract at the dose 400mg/kg showed reaction time  $5.9 \pm 0.52$  ( $P < 0.05$ ). the ethanolic extract possesses anti-inflammatory, anti arthritis and analgesic activity.

**Keywords :** *Merremia emarginata*, anti arthritis, freund's adjuvant, anti inflammatory, analgesic

### INTRODUCTION

Inflammation is a local response of living mammalian tissues to injury. It is a body defense reaction in order to eliminate or limit the speed of injurious agent. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury. Edema formation, leukocyte infiltration and granuloma formation represent such components of inflammation[1]. 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 [2,3]. The pattern of inflammation by freund's adjuvant is similar pattern as arthritis in mammals[4].

Attention is being focused on the investigation of efficacy of plant based drugs used in the traditional medicine because they are economy, have a little side effects and according to W.H.O, about 80% of the world population rely mainly on herbal remedies[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, pcoumaric, ferulic and sinapic acid esters identified in seeds[10].

## MATERIALS AND METHODS

### Preparation of Ethanol Extract

The whole plant *Merremia emarginata* Burm. F were collected from Tirunelveli, Tamilnadu, India during Nov 2011 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 in a soxhlet extractor for 18 hours. The solvent was completely removed by distillation and dried in a vacuum desiccator. The alcoholic extract obtained was screened for anti inflammatory, antiarthritic activity and analgesic activity[11].

### Experimental animals

Colonies inbred strains of Wistar albino rats and Swiss mice of Swiss mice 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 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 for more seven days, we observed the body weight change and toxicity sign for totally fourteen days[13].

### Anti inflammatory activity

The animals were divided into five groups of Six albino wistar rats each (n=6). Acute paw Oedema ( Acute inflammation ) was induced by sub-plantar injection of 0.1% freshly prepared carrageenan suspension in normal saline into the right hind paw of each rat. The left hind paw was injected with same volume of 0.1% of normal saline. Rats were pretreated with Vehicle or EME or Indomethacin 1 hr before carrageenan administration. The paw size was measured in mm using Plethysmometer at 1<sup>st</sup> and 3<sup>rd</sup> hr after carrageenan administration. The percentage inhibition was calculated by following formula[14].

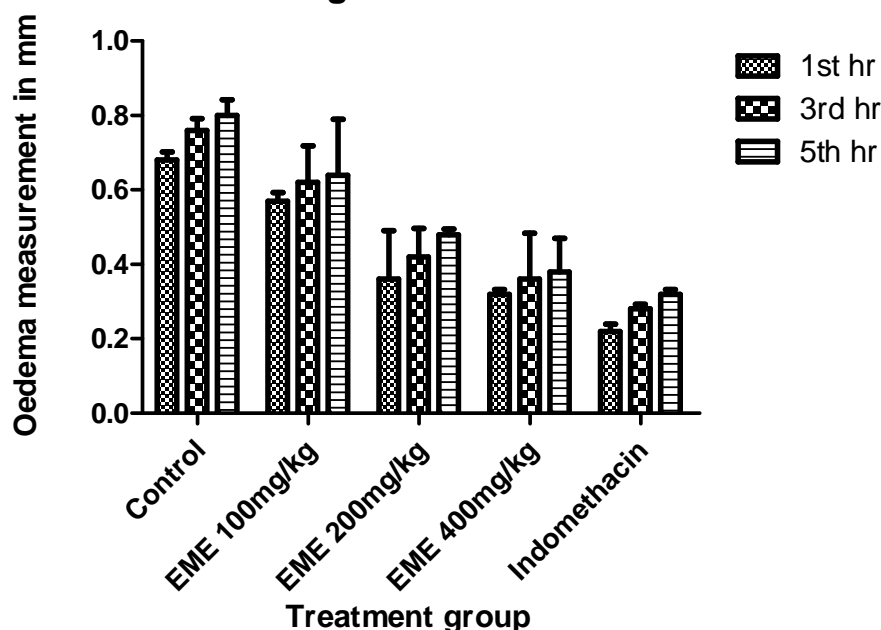
$$\text{Percentage Inhibition} = \frac{V_c - V_t}{V_c} \times 100$$

Where,  $V_c$  = Mean increase in paw volume in control group,  $V_t$  = Mean increase in paw volume in test group

**Anti arthritis activity**

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 tuberculosis* per milliliter sterile paraffin oil into a foot pad of the left hind paw of male rats. The swelling in hind paws were periodically examined in each paw from the ankle using digital Plethysmometer (Panlabs, India). Wistar albino rats of both sex used for the study.

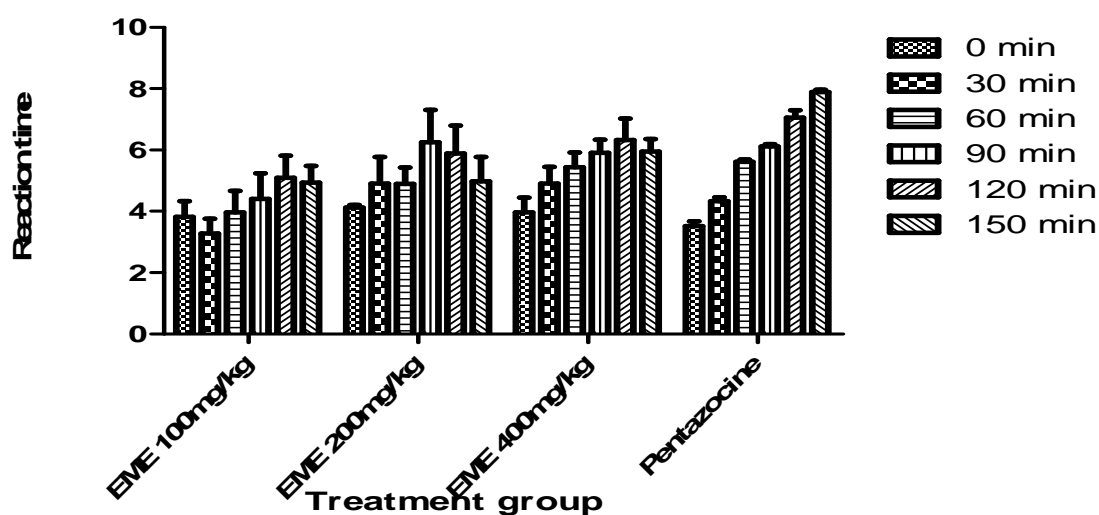
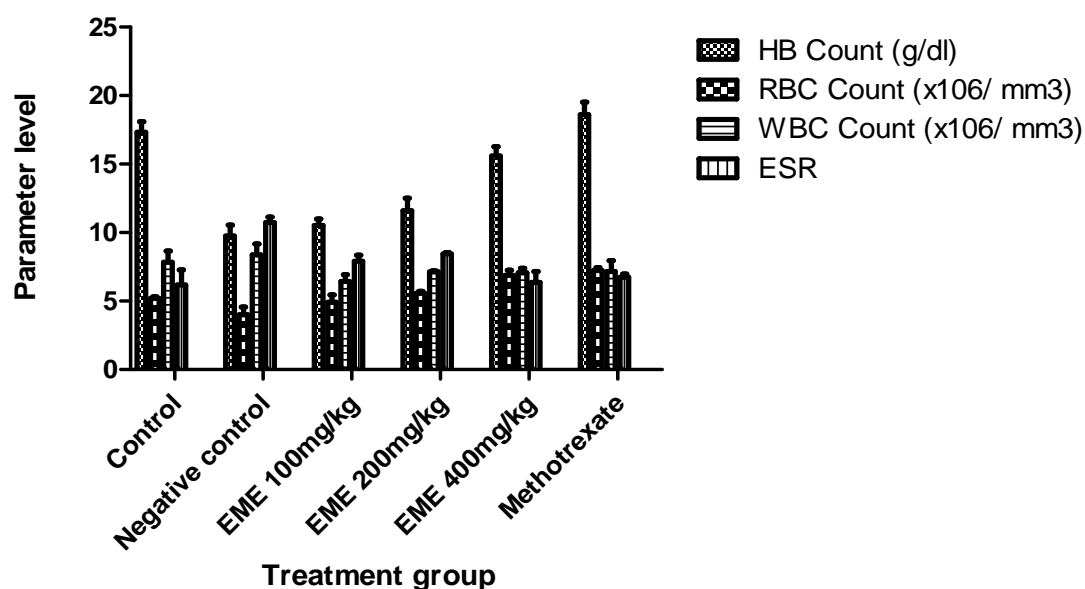
Animals were randomly divided into Six groups of six animals each (n = 6). Group I served as control received 0.1ml of vehicle in which extract is going to be suspended group II served as negative control received 0.1ml freunds adjuvant, and Group III , IV & V received Ethanolic extract of *Merremia emarginata* (EME) at a dose of 100mg/kg, 200 mg/kg and 400 mg/kg. Group VI 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 21<sup>st</sup> day. Paw volume and Paw thickness was measured on 0, 4th, 8th, 14<sup>th</sup> and 21<sup>st</sup> days 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 retro orbital puncture for estimation of serum parameter such as Hb, RBC, WBC and ESR by using various diagnostic kits[16,17].

**Effect of EME in carrageenan induced Paw oedema****Analgesic activity**

The test was conducted on four groups of Six mice each. The mice were placed on hot plate maintained at  $55 \pm 1^\circ\text{C}$  and time in seconds for the paw licking or jumping was recorded as the reaction time. Mice which have shown basal reaction time period below 6 seconds were selected and used for the screening. Mice were fasted for 12 h before test to be started. Mice were pretreated with vehicle or EME or Aspirin (10 mg/kg i.p.). The mouse activity on hot plate was closely observed and the reaction time (latency period) was recorded in seconds as the time taken for the animal to react to the thermal pain by licking its paw or attempting to jump out[18].

**Statistical analysis**

Results were expressed as mean  $\pm$  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 [19].

**RESULTS AND DISCUSSION****Effect of EME in analgesic activity****Effect of EME in antiarthrititis activity**

Acute oral toxicity studies revealed that the both the extracts EME were safe up to a dose level of 2000 mg/kg of body weight (limit test) and LD<sub>50</sub> is more than 2000mg/kg. No lethality or any toxic reactions or moribund state were observed up to the end of the study period.

One-way repeat measure ANOVA showed significant ( $P<0.05$ ) influence of EME on carrageenan-induced inflammation. Dunnett's test indicated EME at 400 mg/kg caused significant ( $p<0.05$ ) decrease in paw oedema compared to vehicle while lower dose 100 mg/kg did not show any effect. Evaluation of the inflammatory status 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. The results obtained for the ethanolic extract of all dose and the standard drug in the complete Freund's adjuvant-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 adjuvant. In the complete Freund adjuvant induced arthritis the dose at 400mg/kg of EME treated animals shows a significant reduction in Hb, WBC and ESR, increase in RBC when compared to the standard drug Methotrexate (5mg/kg) treated animals. Analgesic effect of EME exhibited significant ( $P<0.05$ ) on hot plate reaction time. Dunnett's test showed that for EME at 400 mg/kg showed significant ( $P<0.05$ ) increase in reaction time but the dose of 100 mg/kg did not produce any effect compared to control ( $P>0.05$ ). The effects very much comparable with pentazocin 10 mg/kg that showed increase ( $P<0.05$ ) in reaction time.

### CONCLUSION

From the results observed from the current investigation, it is concluded that the alcoholic extract of *Merremia emarginata* Burm.F possesses potentially useful anti inflammatory, anti arthritic activity and analgesic activity. But the exact mechanism by which *Merremia emarginata* exerts its anti-inflammatory and analgesic activity is not determined yet and needs further investigation to elucidate the other active compounds and underlying mechanism(s).

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### REFERENCES

- [1] Mitchell RN and Cotran RS. In; Robinsons Basic Pathology. 7th edn. Harcourt (India) Pvt. Ltd. New Delhi. **2000**, pp. 33.
- [2] Arends, J. and Dayer, J.M., *Arthritis Rheum.* **1995**, 38, 151-160.
- [3] Saxne, T., Palladino Jr., M.A., Heinegard, D., Talal, N. and Wollheim, F.A., *Arthritis Rheum.* **1988**, 31, 1041-1045.
- [4] Ghosh MN . Fundamentals of experimental pharmacology. Scientific Book Agency. Calcutta. 3rd Edn., **1984**, pp.193-195.
- [5] Dharmasiri JR, Jayakody AC, Galhena G, Liyanage SSP, Rathasooriya WD. *J. Ethnopharmacol.* **2003**; 87:199-206.
- [6] Kirtikar KR, Basu BD, Indian medicinal plants, 2nd ed., Vol. 2, Lalit Mohan basu, Allahabad, India, **1935**, 1(7), pp. 20.
- [7] Shah CS, Sukkawala VM, Chavan AR, Pharmacognostic Study of *Ipomoea reniformis* and Comparison With *Centella Asiatica* Linn, **1962**, 22.
- [8] Nadkarni KM , Indian Material Medica, 3rd ed., Bombay Popular Prakashan, Bombay, **1954**, 690.
- [9] Aswal BS, Goel AK, Mehrotra BN, *Indian J Exp Biol* ,**1984**, 22, 487.
- [10] Usnale SV, *International Journal of Pharmaceutical and Clinical Research*, **2009**, 1(2): 65-67.
- [11] Harbone JB. Phytochemical methods. Chapman and Hall, London. **1998**. pp. 4-32.
- [12] OECD (Organization for economic co-operation and development ) Harmonized integrated hazard classification system for human health and environmental effects of chemical substances. Paris: OECD; **1998**.
- [13] Nimish Pathak, Priyanshee Gohil, Natawar Bhai Patel, Sanjay Kasture, Nirruddin Jivani, Yagnik Bhalodia, *International Journal of Pharmaceutical Sciences and Drug Research*, **2009**, 1(3), 183-187.

- [14] John J.C, Fernandes. J, Nandgude. T, Niphade. S.R, Asavla A, Adeshmukh. P.T. *International Journal of Green Pharmacy*, **2009**, 3(3), 215-219.
- [15] Jaijesh Paval, Srinivasan Keloth Kaitheri, Arunachalam Kumar, Sreejith Govindan, Ciraj Ali Mohammed, Raju Suresh Kumar, Sareesh Naduvil Narayanan, Prasad Alathady Maloor, *Journal of Herbal Medicine and Toxicology*, **2011**, 5(1), 11-16.
- [16] Subhash R. Yende, Vrushali D. Sannapuri, Niraj S. Vyawahare and Uday N. Harle, *International Journal of Pharmaceutical Sciences And Research*, **2008**, 1(9), 129-133.
- [17] Kore K.J, Shete R.V., Desai N.V. *International Journal of Drug Development & Research*, **2011**, 3,(2), 23- 25.
- [18] Jain SC, Jain R, Mascolo N, Capasso F, Vijayvergia R, Sharma RA et al. *Phytotherapy Res.* **1998**; 12 (5): 378-80.
- [19] Kulkarni.S.K, *Handbook of experimental pharmacology*, Vallabh prakashan, **2007**, pp. 172- 189.