



**A COMPARATIVE STUDY OF THE ANTI-ARTHRITIC ACTIVITY OF LEAVES,
STEMS AND ROOTS OF *MIMOSA HAMATA* (WILLD.)**

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ABSTRACT

Adjuvant precipitated arthritis is a persistent crippling, skeleton muscular sickness having nearest approximation to human rheumatoid arthritis for which there may be presently no remedy to be had effecting an everlasting cure. Even current pills used for the amelioration of the symptoms, provide simplest transient remedy and additionally produce intense aspect effects. This paintings turned into aimed to show the conventional declare of *Mimosa hamata* as an anti-arthritic property. In the existing study, anti-arthritic pastime of ethanolic extracts of leaves, stems and roots of *Mimosa hamata* turned into via way of means of Freund's adjuvant precipitated arthritis model. Paw edema, for the duration of arthritis situation turned into corrected on remedy with ethanolic extracts of leaves, stems of *Mimosa hamata* and Aceclofenac. Serum parameters consisting of SGOT, SGPT, ALP, and Total protein had been additionally anticipated for assessing the anti-arthritic capacity of ethanolic extracts of leaves, stems and roots of *Mimosa hamata*. Biochemical parameters consisting of hemoglobin and erythrocyte sedimentation rate (ESR), WBC, RBC had been anticipated. The effects of the modern research concluded, ethanolic extracts of stems of *Mimosa hamata* own a substantial anti-arthritic pastime in opposition to adjuvant precipitated arthritis and

justifying its healing position in arthritic situation. The located anti-arthritic pastime can be because of the presence of phytoconstituents consisting of flavonoids and tannins.

Keywords: *Mimosa hamata*, anti-arthritic activity, Complete Freund's adjuvant, SGOT, SGPT, ALP

INTRODUCTION

Rheumatoid arthritis (RA) may be a chronic autoimmune disorder characterized by joint swelling, synovial inflammation and cartilage destruction and commonly cause significant disability [1]. It affects about 1% of the population of world during a female and male ratio of two.5:1. It caused by number of pro-inflammatory molecules released by macrophages including Species of reactive oxygen and eicosanoids such as prostaglandins, leukotrienes and cytokines The regulation of certain mediators secreted by macrophages and other immune cells and the control of arachidonic acid metabolism by inhibiting enzymes such as COX and LOX are possible targets for chronic inflammatory conditions [2]. Even though various categories like immunosuppressants, NSAIDs, steroidal anti-inflammatory drugs are getting used till now, the potential side effects provide a limitation for his or her use [3]. Now it's a growing concern all over for the event of latest safe, potent, less toxic anti-arthritis drug. Hence, there's a requirement to look for more naturally available alternatives, in order that their therapeutic values are often assessed and expanded. Plants are one

among the foremost important sources of medicines. India is understood because the "Emporium of Medicinal plants" thanks to availability of several thousands of medicinal plants within the different bioclimatic zones anti-inflammatory diseases including atrophic arthritis are still one among the most health problems of the world's population. Plant drugs are known to play a vital role in management of inflammatory diseases [4].

Genus *Mimosa* (family: Mimosaceae) has about 400 species which are mainly shrubs & small trees in tropics. About 8 species are found in India, with medicinal importance [5] while some are of ornamental use. *Mimosa pudica* the curious plant in the genus is a creeping form. Because of the way it folds its leaves when touched, it is known as touch-me-not plant. *Mimosa hamata* also folds its leaves when touched [6]. *Mimosa hamata* is a much straggling shrub occurring in tropics & widely distributed in India & Pakistan [7]. The plant is used for urinary complaints & as a tonic against general weakness. A paste of leaves is applied over glandular swellings & is used in dressing for sinus, sores & piles [8]. Its roots possess

contraceptive efficacy while seeds are used as blood purifier [9]. Various bioefficacies viz., antifungal activity of deprotenized leaf extract [10, 11]. Antibacterial activity of alcoholic extract of aerial parts, antiviral activity of methanolic extract of roots⁵ and Antioxidant activity [12] have been reported.

The major phytoconstituents present in *Mimosa hamata* (Willd.) include 4-ethylgallic acid from fresh flowers, triterpenesaponin B (3-O-Larabiosyl-D-glucosyl morolic acid), mimonoside A, B, C and saponin A (3-O-D-glucosyl-L-rhamnosyl morolic acid) from the roots, ethylgallate and gallic acid from leaves [13].

Mimosa hamata (Willd.) belonging to the family Mimosaceae is being selected for phytochemical investigations to pin point various pharmacological activities. *Mimosa hamata* has been reported to possess antibacterial, antiviral & antioxidant properties [5, 10, 11, 12]. It was found that no substantial work of *Mimosa hamata* was carried out for its anti-arthritis activity. As reported previously that *Mimosa hamata* possess anti-oxidant activity using that as a basis we have moved towards evaluating the anti-arthritis potential of the plant. To investigate new lead molecules having biological activities such as anti-arthritis for the welfare of mankind. It will help to produce novel

drugs having no side-effect or less side-effect and less cost than synthetic drug.

MATERIALS AND METHODS

Plant material

For this study roots, stems and leaves of *M. hamata* plant was collected from Methwade, Tal. Sangola, Dist. Solapur (Maharashtra) and was authenticated at Botanical Survey of India, Pune. The specimen of the voucher will be deposited within the institution and in the Botany Department of 'Herbarium,' Solapur University, Solapur during the month of September 2015. The material was dried under shade at a temperature of about 15 days. The dried plant samples were powdered by a mechanical grinder and sifted with a particle size of 40-100 mm. The powder was stored in polythene bags at temperature prior to extraction.

Preparation of Alcoholic extract:

The collected roots, stems and leaves were shade dried, reduced to a rough powder during a mechanical grinder to get of desired particle size (40# sieve). About 200 gms of powdered material was subjected to exhaustive extraction with 90% alcohol during a Soxhlet extractor at a temperature of 60 – 70°C, targeting a rotary flash evaporator at 50°C (Superfit, India), and eventually to dry powder. Some a part of the entire extract was reserved for phytochemical investigation and remainder

of the extract was used for biological activity.

Preliminary phytochemical analysis:

The ethanolic extract was then subjected to preliminary phytochemical analysis to assess the presence of various phytoconstituents, it revealed the presence of flavonoids, carbohydrate, glycosides and tannins. Preliminary Thin layer chromatography studies also confirmed these constituents [14].

Animals: Wistar albino rats weighing 175-225g of either sex maintained under standard husbandry conditions (temp $23\pm 2^{\circ}\text{C}$, relative humidity $55\pm 10\%$ and 12 hours light dark cycle) were used for the screening. Animals were fed with standard laboratory food and ad libitum during the study period. The experiments were performed after the experimental protocols approved by the institutional animal ethics committee, India 2009.

Acute oral toxicity studies [15]:- Acute oral toxicity study was performed to ascertain safe dose of the extract by the acute oral toxic class method by the Organization of Economic Cooperation and Development (OECD) 423 guidelines.

Evaluation of In vivo Anti-Arthritic Activity:-

Complete Freund's adjuvant induced arthritis [16]

The method according to Pearson and Wood et.al; has been adopted for evaluation of anti-arthritic property. Freund's adjuvant induced Arthritis model was used to assess the anti-arthritic activity in Albino rats. Animals were randomly divided into nine groups of six animals each.

Arthritis was induced by the intra-dermal injection of 0.1 ml of Freund's complete adjuvant (FCA, Sigma) in the sub-plantar region of the right hind paw on day 0. The rats were divided into nine groups of six rats each as follows:

Group 1 - non-arthritic, vehicle control (1 ml of normal saline, p.o.),

Group 2 - arthritic, vehicle control (CFA+1 ml of normal saline, p.o.),

Group 3 - arthritic animals treated with standard (CFA+ Aceclofenac 10 mg/kg, p.o.),

Group 4 - arthritic animals treated with test drug (CFA+ELMH 200 mg/kg, p.o.),

Group 5 - arthritic animals treated with test drug (CFA+ELMH 400 mg/kg, p.o.),

Group 6 - arthritic animals treated with test drug (CFA+ESMH 200 mg/kg, p.o.),

Group 7 - arthritic animals treated with test drug (CFA+ESMH 400 mg/kg, p.o.),

Group 8 - arthritic animals treated with test drug (CFA+ERMH 200 mg/kg, p.o.),

Group 9 - arthritic animals treated with test drug (CFA+ERMH 400 mg/kg, p.o.).

Drug treatment started on the first day (0 day), 30 min before the adjuvant injection and continued until the 21st day. Paw volume was measured on the 4th, 8th, 14th and 21st day using a plethysmometer. Half of the inhibition of paw volume of the injected paw over the vehicle control at day 4,8,14 and 21 was evaluated using the following formula.

$$i = \frac{[(V) \text{ control} - (V) \text{ treated}] \times 100}{(V) \text{ Control}}$$

(V) Control

Where i = % inhibition of paw oedema

V treated = Paw volume of treated rat

V untreated = Paw volume untreated rat

The rats were anaesthetized under light ether anesthesia and blood was collected by

retro-orbital puncture for estimation of serum parameter such as SGOT, SGPT, ALP and Total protein by using various diagnostic kits.

Table 1: Effect of ethanolic extracts of leaves, stems and roots of *Mimosa hamata* on paw volume of arthritic rats

Groups	Paw volume (ml ± SEM) in rats					% inhibition of paw oedema On 21st day
	0 th day	4 th day	8 th day	14 th day	21 st day	
Negative Control	0.31±0.08	0.32±0.12	0.31±0.05	0.28±0.07	0.23±0.04	
Positive Control	0.33±0.10	0.78±0.08	0.75±0.09	0.70±0.04	0.73±0.07	
Acceclofenac sodium (10mg/kg)	0.36±0.13 (↓9.090%)	0.41±0.05*** (↓47.435%)	0.38±0.02*** (↓49.333%)	0.40±0.05*** (↓42.857%)	0.31±0.04*** (↓57.534%)	57.53%
ELMH (200 mg/kg)	0.31±0.07 (↓6.060%)	0.51±0.05* (↓34.615%)	0.43±0.03* (↓42.666%)	0.41±0.02* (↓41.428%)	0.39±0.05* (↓46.575%)	46.57%
ELMH (400 mg/kg)	0.32±0.06 (↓3.030%)	0.35±0.07** (↓55.128%)	0.37±0.08** (↓50.666%)	0.39±0.06** (↓44.286%)	0.38±0.05** (↓47.945%)	47.945%
ESMH (200 mg/kg)	0.30±0.05 (↓9.090%)	0.31±0.05** (↓60.25%)	0.36±0.06** (↓52.00%)	0.32±0.04** (↓54.286%)	0.34±0.04** (↓53.424%)	53.42%
ESMH (400 mg/kg)	0.32±0.06 (↓3.030%)	0.34±0.03*** (↓56.410%)	0.37±0.06*** (↓50.666%)	0.35±0.04*** (↓50.00%)	0.33±0.01*** (↓54.794%)	54.79%
ERMH (200 mg/kg)	0.32±0.08 (↓3.030%)	0.47±0.08** (↓39.743%)	0.48±0.07** (↓36.00%)	0.45±0.06** (↓35.714%)	0.40±0.05** (↓45.205%)	45.20%
ERMH (400 mg/kg)	0.31±0.04 (↓6.060%)	0.37±0.04** (↓52.564%)	0.39±0.06** (↓48.00%)	0.41±0.06** (↓41.428%)	0.36±0.05** (↓50.684%)	50.68%

Values are expressed as mean ± S. E. M. (n= 6) Statistical analysis was carried out by ANOVA followed by the Dunnet’s test at the significance level of *p<0.05; **p<0.01; *** p<0.001, as compared with positive (arthritic) control. Values in bracket indicate decrease as compared to positive control.

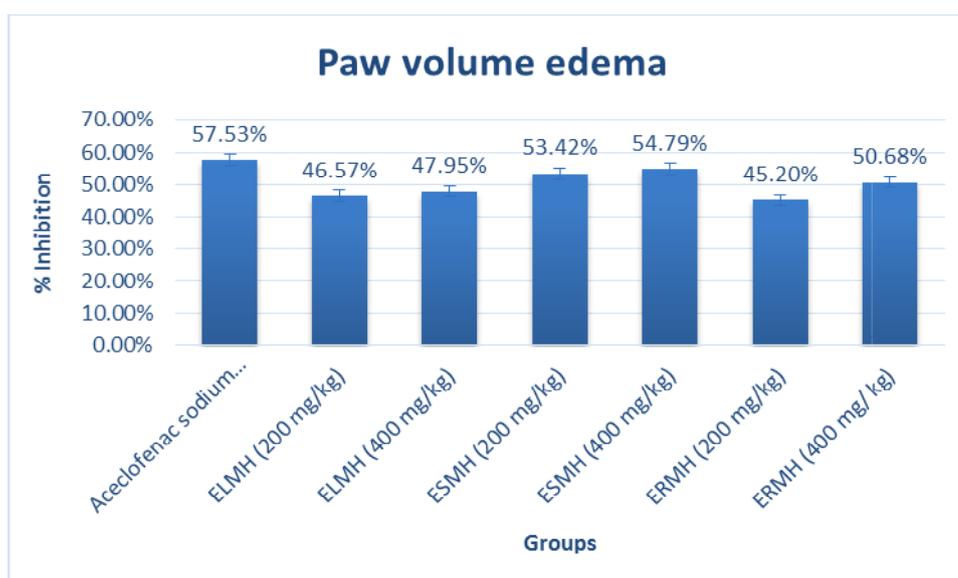


Figure 1: Comparisons of effect of ethanolic extracts of leaves, stems and roots of *Mimosa hamata* on paw volume of arthritic rats

ELMH- ethanolic extract of leaves, ESMH- ethanolic extract of stems, ERMH- ethanolic extract of roots

Table 2: Effect of ethanolic extracts of *M. hamata* extracts on FCA-induced changes in hematological profile

Hematological Profile				
Treatment (Dose mg/kg)	Hemoglobin g/dl	ESR mm/h	WBC $\times 10^3/\text{mm}^3$	RBC $\times 10^6/\text{mm}^3$
Non arthritic control	14.13 \pm 0.604	3.817 \pm 0.142	10.37 \pm 0.436	7.014 \pm 0.352
Arthritic control	11.58 \pm 0.292	12.42 \pm 0.86	11.98 \pm 0.632	6.861 \pm 0.563
Aceclofenac (10mg/kg)	13.50 \pm 0.183***	5.750 \pm 0.519***	10.65 \pm 0.489***	6.800 \pm 0.139***
ELMH 200	13.95 \pm 0.272	5.883 \pm 0.364	10.53 \pm 0.670	7.150 \pm 0.397
ELMH 400	13.80 \pm 0.198	5.683 \pm 0.233	10.75 \pm 0.606	6.933 \pm 0.2459
ESMH 200	13.45 \pm 0.263**	6.783 \pm 0.309**	11.98 \pm 0.563**	7.067 \pm 0.2231**
ESMH 400	13.62 \pm 0.268***	5.650 \pm 0.291***	10.85 \pm 0.504***	7.033 \pm 0.2246***
ERMH 200	13.45 \pm 0.280	5.767 \pm 0.315	10.65 \pm 0.408	6.683 \pm 0.3842
ERMH 400	11.82 \pm 0.605	8.317 \pm 0.617	11.80 \pm 0.619	6.567 \pm 0.346

Values are expressed as mean \pm S. E. M. (n= 6) Statistical analysis was carried out by ANOVA followed by the Dunnet's test at the significance level of *p<0.05; **p<0.01; *** p <0.001, as compared with positive (arthritic) control

Table 3:- Effect of ethanolic extracts of *M. hamata* on FCA-induced changes in biochemistry parameters

Treatment (Dose mg/kg)	SGOT (IU/L)	SGPT (IU/L)	Total Proteins (gm%)	ALP (mg/dl)
Non arthritic control	57.37 \pm 1.200	62.14 \pm 3.601	6.86 \pm 0.2379	5.36 \pm 0.040
Arthritic control (10mg/kg)	56.59 \pm 0.972	65.40 \pm 4.44	6.75 \pm 0.318	5.375 \pm 0.044
Aceclofenac	56.93 \pm 3.99***	62.73 \pm 1.926***	6.76 \pm 0.369***	5.596 \pm 0.221***
ELMH 200	56.93 \pm 2.03*	64.06 \pm 3.232*	6.60 \pm 0.222*	5.701 \pm 0.223*
ELMH 400	58.93 \pm 2.792*	64.51 \pm 1.184*	7.20 \pm 0.203*	5.725 \pm 0.2229*
ESMH 200	56.59 \pm 1.619**	61.73 \pm 3.129*	6.63 \pm 0.186**	5.740 \pm 0.2164**
ESMH 400	56.26 \pm 3.074***	61.00 \pm 2.778*	6.75 \pm 0.122***	5.940 \pm 0.3518***
ERMH 200	58.26 \pm 1.722*	61.65 \pm 2.584*	6.75 \pm 0.223*	5.507 \pm 0.2014*
ERMH 400	57.76 \pm 2.795**	64.81 \pm 1.015*	6.75 \pm 0.172**	5.580 \pm 0.247**

Values are expressed as mean \pm S. E. M. (n= 6) Statistical analysis was carried out by ANOVA followed by the Dunnet's test at the significance level of *p<0.05; **p<0.01; *** p <0.001, as compared with positive (arthritic) control

RESULTS

Preliminary phytochemical screening of ethanolic extract of the leaves, stems and roots of *Mimosa hamata* (Willd.) revealed the presence of bioactive components like flavonoids, carbohydrate, glycosides and tannins.

FCA-induced rats paw edema:

Induction of FCA caused significant (P<0.001) increase in paw volume of injected right hind paw and maximum increase in paw volume was noted on day 21 which was recorded as change in paw volume 0.73 \pm 0.07ml. Significant (P<0.001) increase in paw volume of all FCA injected

rats was evident. The rise in paw volume progressed till day 21 in arthritic group. Treatment of Aceclofenac (10 mg/kg) showed significant (P<0.001) decrease within the paw volume of right hind paw at day 14th and 21st with the percent inhibition of 42.857% and 57.534%, respectively.

Administration of 400 mg/kg of ELMH showed significant (P<0.01) decrease in the paw volume of right hind paw at day 21, showing 47.945% of inhibition, while administration of 200 mg/kg of ELMH showed significant (P<0.05) decrease in the paw volume of right hind paw at day 21, showing 46.575 of inhibition.

Administration of 400 mg/kg of ESMH showed significant ($P < 0.001$) decrease in the paw volume of right hind paw at day 21, showing 54.794% of inhibition, respectively, while administration of 200 mg/kg of ESMH showed significant ($P < 0.01$) decrease in the paw volume of right hind paw at day 21, showing 53.42% of inhibition.

Administration of 400 mg/kg of ERMH showed significant ($P < 0.01$) decrease in the paw volume of right hind paw at day 21, showing 50.684% of inhibition, respectively, while administration of 200 mg/kg of ERMH showed significant ($P < 0.01$) decrease in the paw volume of right hind paw at day 21, showing 45.20% of inhibition.

Administration of 400 mg/kg of ESMH showed significant ($P < 0.001$) decrease in the paw volume of right hind paw at day 21, showing 54.79% of inhibition, respectively, while administration of 200 mg/kg of ESMH showed significant ($P < 0.001$) decrease in the paw volume of right hind paw at day 21, showing 53.42% of inhibition. It was concluded that the ethanolic extract of stems of *Mimosa hamata* 400mg/kg showed significant inhibition of right hind paw volume compared to other groups as shown in **Table 1**

However there was no significant change in the paw volume of the left hind paw of all

arthritic rats which was kept as normal control.

Biochemistry profiles:-

No abnormalities were found within the biochemical changes, FCA-injected group slight reduced SGOT and total proteins. Slight elevated the SGPT compared with normal group. There was no significant difference between treated group and control group. The treatment with ethanolic extract of stems of *Mimosa hamata* 400 mg/kg group showed significant prevention of the elevation of the serum SGOT, ALP and total proteins, whereas decrease within the SGPT level as compared to regulate group. These results confirm that there was no toxic effect found on liver and kidney (**Table 3**).

Hematological profiles:-

FCA-induced arthritic rats at 21st days showed slight elevation in the total WBC count and reduction in RBC. However, significant ($p < 0.001$) increased ESR while the hemoglobin was significant ($p < 0.001$) reduced in the control group when compared with normal group. The treatment with ethanolic extract of stems of *Mimosa hamata* 400 mg/kg treated groups recovery were observed in RBC and WBC count however, significant ($p < 0.001$) recovery in Hemoglobin content, ESR level compared with the control group (**Table 2**).

DISCUSSION

Rheumatoid arthritis may be a systemic autoimmune disorder characterized by articular inflammation that eventually results in the destruction of joints. As mentioned within the above results it had been concluded that, the ethanolic extracts of stems of *Mimosa hamata* 400 mg/kg suppressed the joint inflammation and destruction in adjuvant arthritic rats. It also normalized the hematological parameters and serum enzyme levels in arthritic rats treated with ethanolic extract of leaves, stem and roots of *Mimosa hamata* in comparison with arthritic control. The ethanolic extracts of stems 400 mg/kg of *Mimosa hamata* (Willd.) is an efficient anti-arthritic agent experimentally compared to the leaves and roots of *Mimosa hamata* (Willd.) and holds prospect in future atrophic arthritis treatment. Though, exact mechanism of repressing the arthritic state by ethanolic extracts of stems 400 mg/kg *Mimosa hamata* isn't identified, its beneficial effects on RA could possibly be correlated with the presence phyto-constituents like flavonoids, tannins, glycosides and carbohydrates.

CONCLUSION

This contemporary research lends pharmacological support to reported folkloric usage of stems of *Mimosa hamata* in the treatment and management of painful arthritic inflammatory conditions. Based on

the results, further thorough studies are required for appraisal of exact mechanism of action of *Mimosa hamata*, isolation of active constituents that could conclusively establish stems of *Mimosa hamata* as a potentially safer disease modifying agent in the treatment of RA.

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Conflict of interest statement

We declare that we have no conflict of interest.

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