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ANTI ARTHRITIC AVTIVITY OF LEAVES OF *MERREMIA EMARGINATA*

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ABSTRACT

The anti arthritic activity of various extracts of *Merremia emarginata* is evaluated by using freund's adjuvant induced arthritic and formaldehyde induced arthritic model. To ensure the safety of the drug, acute toxicity study is also carried out. The extracts had shown a significant anti arthritic activity in both the model by reducing the increased paw volume and also by restoring the biochemical parameters to its normal levels.

Key words: *Merremia emarginata*, Arthritic, Freund's adjuvant, Radiology, Formaldehyde.

INTRODUCTION

Rheumatoid Arthritis is a chronic autoimmune disorder characterized by various symptoms such as inflammation with pain over joints, swelling, redness and morning stiffness. Although the cause for the disorder is unknown, the autoimmunity plays a vital role. The treatment includes NSAIDS, DMARDs and corticosteroids but results in many serious side effects on GIT & Cardiac which then affects the other systems[8]. Keeping the above points in view, we had evaluated the anti arthritic activity of our plant in various animal models which may be useful for the society if proved.

Procedure

The plant specimen for the present study was collected from valayampattu, Tamilnadu. The leaves were dried and powdered. It was extracted by cold maceration process in Ethanol and water for 48hrs. The Extracts thus obtained were used for the study.

Anti arthritic activity [4-7]

Freunds adjuvant induced arthritis

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Freund's adjuvant induced arthritis model is used to assess the anti-arthritic activity in albino rats. Healthy young adult Swiss albino rats of either sex weighing 200gm of animals were taken and divided into five groups of four animals each. Group I serve as control which receives 5ml/kg saline, Group II receives prednisolone 10 mg/kg po., respectively and Group III – V receives single dose of the samples. Drug treatment was started from the day of adjuvant injection (0 day-30 min before injection) and continued till 21st day. Paw volume was measured on 7th and 21st day with the help of a volume transducer attached with strain gage coupler of student physiograph. The percentage increase in paw edema with respect to initial paw volume was calculated on respective days in percentage. The reduction in percentage of increased paw volume showed the higher protection activity.

Formaldehyde induced arthritis

Five groups of male Wistar albino rats (n=3) were used for this study. Baseline recording of the joint diameter is made by using a micrometer screw gauge. Drugs/vehicle was administered for duration of 10 days. Thirty minutes after administration of vehicle/drugs, arthritis was induced by sub plantar administration of 0.1 ml formaldehyde (2% v/v) into the left hind paw of all the animals on days 1, 3, 7 & 8. Increase in joint diameter

of the injected paw was measured on days 8, 9 and10th day, 30 mins after administration of the respective vehicle/drug treatment.

Control group 1: Formaldehyde+ 2% Tween 80 (10 ml /kg b.w)

Standard group 2: Formaldehyde + Diclofenac Sodium (10 mg/kg b.w)

Test group 1: Formaldehyde + Extract (Dose-I mg/kg b.w)

Test group 2: Formaldehyde + Fraction (Dose-II mg/kg b.w)

Test group 3: Formaldehyde + Isolated Compound (Dose-I mg/kg b.w)

RESULTS

Anti Arthritic activity

BODY WEIGHT

In the present study, it is clear from the data obtained that there is a close relationship between the extent of joint inflammation and the degree of weight loss. The induced control group when compared to the standard and extract treated groups; it was found that the weight of the rats was highest in case of the group VI. Standard drug, EAFME and ICME significantly ($p<0.01$) increased the body weight of the animal as compared to induced control group on 14^{th} & 28^{th} day but MEME non-significant on 14^{th} day, as depicted in [Table 1]. The plant showed a fractionation dependant increase in the body weight of the rats.

An increase in Paw volume was seen in all animals throughout the observation period. Maximum Paw volume was observed on day 21, after which there was a gradual decrease except in the induced control and MEME treated groups, which showed a increase in Paw volume from Day 1 to day 28. Although all drug treated groups showed a decrease in joint swelling as compared to the induced control, the difference was significant ($p<0.01$) in Group III, V, VI on all observation days. MEME at a dose of 500 mg/kg (Group IV) produced a non significant reduction in paw volume on all observation days.

Blood and serum analysis

As a result of inflammation induced by formaldehyde, the levels of Hb mg/dl & ESR mm/hr were increased in all arthritic rats as compared to induced control rats. After treatment, the levels of these haematological parameters were significantly ($p<0.01$) decreased in group V & VI rats as compared to induced control rats except Group IV revealed non-significant. ICME treated group prevented haematological changes to a greater extent than the Diclofenac sodium (10 mg/kg). However, treated groups III, V & VI serum shows RA factor negative as compared to induced control, which shows positive. The group IV shows RA factor positive, the group V & VI proving its anti-arthritic efficacy.

Administration of 0.1ml of 2% v/v formaldehyde produced an increase in the joint diameter of all the animals, which was persistent throughout the observation period (Fig. 2). Maximum joint swelling was observed on day 14, after which there was a gradual decrease except in the induced control and MEME treated groups, which showed an increase in joint diameter from Day 1 to day 28. The ICME was more efficacious than Diclofenac in reducing the joint swelling.

RADIOLOGICAL STUDIES

The radiographic features of the rat joints in formaldehyde induced arthritic model are shown in figure 13.15, 16, 17, 18, 19 & 20. In formaldehyde induced arthritic rat (group II), soft tissue swelling along with narrowing of the joint spaces were observed which implies the bony destruction in arthritic condition. The standard drug Diclofenac sodium treated groups have prevented this bony destruction and also there is decreased swelling of the joint. The EAFME & ICME treatment for 28 days have shown significant prevention against bony destruction by showing less soft tissue swelling and narrowing of joint spaces when compared with induced control.

HISTOPATHOLOGICAL STUDIES

Histopathological studies of ankle joint reports confirmed that there is severe bone erosion with presence of neutrophil infiltration and pannus formation in control group (Fig). The treatment groups EAFME (Fig) and ICME revealed reduction in pannus formation and bone resorption, joint inflammation with reduced neutrophil infiltration. Among the two ICME proved anti-arthritic action that is comparable to that of standard diclofenac group (Fig). The MEME slide shows moderate level of cells and mild effect on inflammation.

Table 1. Effect of extract on body weight of the animals

Groups	Treatment and Dose	Body Weight (gms)		
		0 day	14^{th} day	28^{th} day
I	Normal Control	153.5±1.99	167.83±3.52	175.66±2.12
II	Induced Control	148.33±3.05	157.66±3.11	164.5±3.9
III	Diclofenac (10mg/kg)	154.25±1.51	170.32±2**	190.25±1.86**
IV	MEME	151.66±3.3	164.6±3.99^{ns}	175.66±3.18**
V	EAFME	153.33±3.57	167.66±2.78*	182.62±3.10**

VI	ICME	155.83+2.71	178.33+2.47**	188.24+1.83**

All values are Mean±SEM. Statistical analysis by One-way ANOVA followed by Dunnett's Multiple Comparison.
[ns] non-significant, *P<0.05 ,**P<0.01 as compared to induced control.

Table 2. Effect of extracts on Hb, ESR & RA factor

Groups	Treatment & Dose	Hb mg/dl	ESR mm/hr	RA factor
I	Normal Control	16.8±1.25	12.28±0.54	Negative
II	Induced Control	8.51±2.34	24.36±1.49	Positive
III	Diclofenac (10mg/kg)	15.21±3.54**	9.62±0.42**	Negative
IV	MEME	10.37±2.37[ns]	22.41±0.86[ns]	Positive
V	EAFME	13.86±4.21**	8.28±0.57**	Negative
VI	ICME	15.26±3.28**	10.92±0.21**	Negative

All values are Mean±SEM. Statistical analysis by One-way ANOVA followed by Dunnett's Multiple Comparison.
[ns] non-significant, P<0.01 as compared to induced control.

Table 3. Effect of extracts & fractions on joint swelling (Paw volume)

Groups	Treatment & Dose	Paw volume in ml					% inhibition of paw volume on 28th day
		1st day	7th day	14th day	21st day	28th day	
I	Normal Control	0.04±0.00	0.04±0.00	0.05±0.00	0.05±0.00	0.04±0.00	-
II	Induced Control	0.26±0.01	0.46±0.01	0.55±0.02	0.6±0.02	0.67±0.01	0
III	Diclofenac (10mg/kg)	0.20±.02**	0.31±0.03**	0.34±0.01**	0.38±0.02**	0.30±0.01**	55.2
IV	MEME	0.22±0.02[ns]	0.43±0.02[ns]	0.50±0.02[ns]	0.54±0.02[ns]	0.61±0.01[ns]	8.9
V	EAFME	0.21±0.00**	0.37±0.01**	0.38±0.00**	0.42±0.02**	0.35±0.02**	47.86
VI	ICME	0.18±0.00**	0.30±0.02**	0.35±0.01**	0.37±0.01**	0.29±0.02**	56.71

All values are Mean±SEM. Statistical analysis by One-way ANOVA followed by Dunnett's Multiple Comparison.
[ns] non-significant, P<0.01 as compared to induced control.

Table 4. Effect of extracts on joint diameter on various groups

Groups	Treatment & Dose	Joint diameter in mm					% decrease of joint diameter 28th day
		1st day	7th day	14th day	21st day	28th day	
1	Normal Control	0.42±0.02	0.42±0.02	0.42±0.02	0.42±0.01	0.43±0.01	-
2	Induced Control	0.45±0.02	0.69±0.01	0.77±0.01	0.81±0.02	0.89±0.02	0
3	Diclofenac (10mg/kg)	0.40±0.03	0.42±0.02	0.51±0.03	0.47±0.02	0.45±0.02	49.43
4	MEME	0.42±0.02	0.58±0.02	0.67±0.01	0.72±0.02	0.75±0.01	15.73
5	EAFME	0.43±0.01	0.48±0.01	0.56±0.01	0.52±0.03	0.50±0.03	43.82
6	ICME	0.42±0.01	0.45±0.02	0.49±0.02	0.45±0.02	0.42±0.02	52.80

Figure 1: Graph showing the effect of extracts on body weight of animals

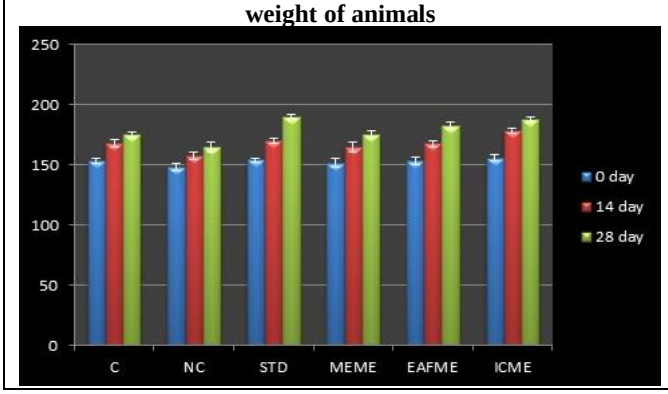


Figure 2: Effect of *Merremia emarginata* on joint swelling (Paw volume) in formaldehyde induced arthritis

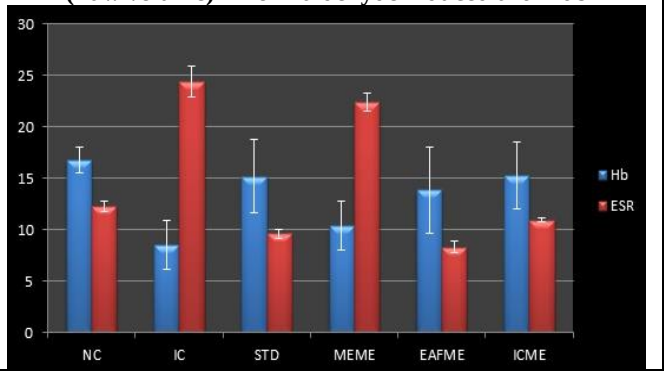


Figure 3: Effect of extracts & fractions on joint diameter in formaldehyde induced arthritis

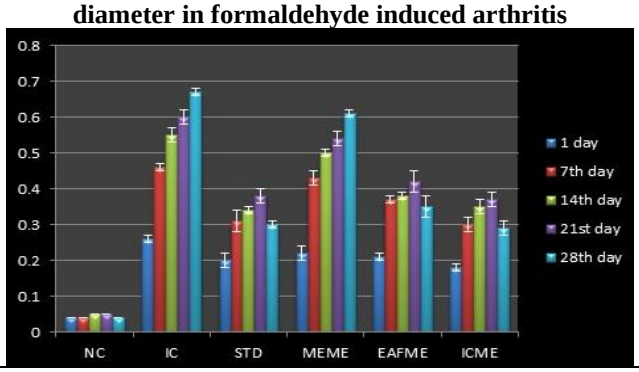


Figure 4: Graph showing the joint diameter of various groups

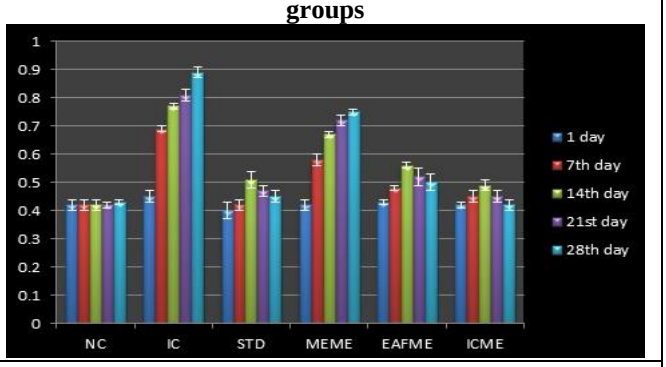


Figure 5: Normal Control

Figure 6: Negative control

Figure 7: Standard (Diclofenac 10mg/kg)

Figure 8: MEME

Figure 9: EAFME

Figure 10: ICME

Fig 11: Normal Control DMSO 2ml/kg

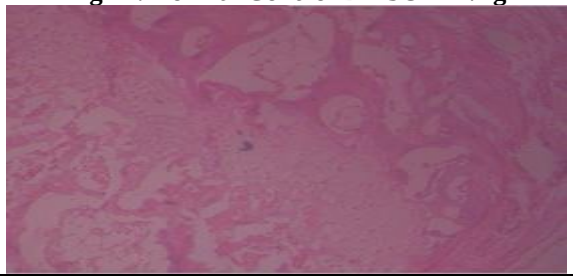


Fig 12:Negative control

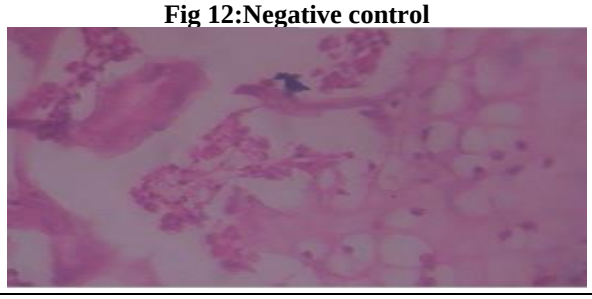


Fig 13: Standard (Diclofenac 10mg/kg)

Fig 14: MEME

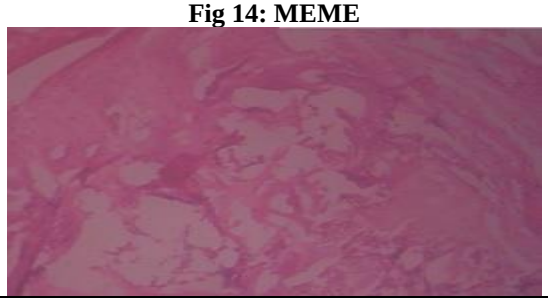


Fig 15:EAFME

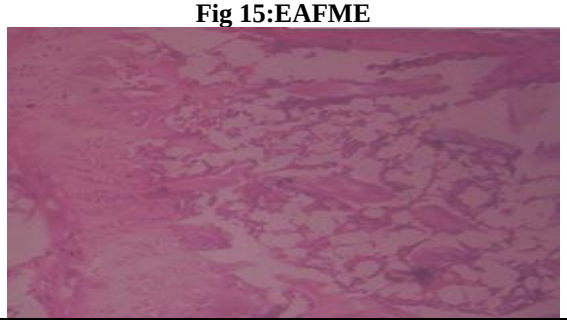
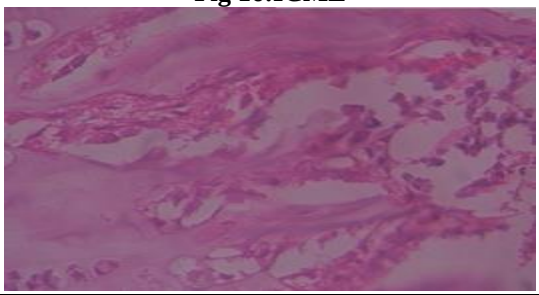


Fig 16:ICME



DISCUSSION

By using Formaldehyde induced and freund's adjuvant induced arthritic model, the potential of the extract and fraction about its anti arthritic activity has been carried out.

The formalin test is a very useful method for not only assessing the antinociceptive antinociceptive drugs but also helping in the elucidation of the action mechanism. The neurogenic phase is probably a di rect result of stimulation in the paw and reflects centrally mediated pain with release of substances while the late phase is due to the release of histamine, serotonin & bradykinin. The ethyl acetate fraction does not reduced the paw volume in the initial period of the treatment revealing that it is not acting by reducing the levels of histamine and serotonin but had reduced the paw volume in the later period of treatment confirming that it is acting by reducing the COX induced prostraglandins.

Complete Freund's adjuvant induced arthritis is one of the most widely used models as it has been shown to share a number of clinical and immunological features with human arthritis. Therefore, this model is used with a relatively high degree of validity for evaluating agents with potential antiarthritic activity. In the vehicle treated animals (control), there was an increase in the joint diameter after day 14, which can be attributed to the delayed immunological flare in the disease. However, the increase in joint diameter was not observed in the drug treated groups, suggesting the involvement of mechanisms other than inhibition of inflammatory autocoids in the antiarthritic activity of the test drug [9].

From the result, it is clear that the decrease in the Hemoglobin levels is an indication of anaemia which may be due to the improper storage of iron in the reticuloendothelial system and synovial joints. The ESR count which significantly increased in the arthritic control group had gets reduced in the extract treated group. Than the methanolic extract, the ethyl acetate fraction and the isolated compound had shown a significant and potent anti arthritic activity by reducing the swelling, increasing the body weight, Haemoglobin levels and restoring ESR level. The results have also been well supported by the radiological studies. From the radiological study, it may also be known that the leukocyte count of the drug treated rats may be restored to its normal levels which may be reason for the protection of joints in those animal groups.

CONCLUSION

Hence the plant *Merremia emarginata* can be investigated further which may yield us a potent anti arthritic drug which will be useful for our society to eradicate the disease.

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