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Original Article

Anti-Arthritic Activity of *Rivea hypocrateriformis* Leaf Extract on Freund's Adjuvant Induced Arthritis in Rats

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ARTICLE INFO	ABSTRACT

Rivea hypocrateriformis is a plant traditionally used by people of Karnataka to cure various types of diseases such as malaria and to relieve pain. Its anti-oxidant, analgesic and anti-inflammatory activities have been reported, but literature survey reveals that its anti-arthritic activity has not yet been investigated. Hence, the present study was undertaken with the objective of evaluating the anti-arthritic activity of methanolic extract of leaves of *Rivea hypocrateriformis*. **Material and methods**: Freund's complete adjuvant was injected into the left hind paw of rats and methanolic extract of leaves of *Rivea hypocrateriformis* (250 and 500 mg/kg; p.o) was administered for 21 days. Body weight of all the rats of all groups was recorded initially and at the end of treatment period of 21 days. Paw volume was measured plethysmographically on 1st, 4th, 8th, 14th and 21st day of drug treatment. On completion of treatment period, blood sample was collected from retro-orbital plexus of all rats of all groups for evaluation of various biochemical and hematological parameters as major markers of arthritis and Arthritic Index was calculated. **Results and Discussion:** Freund's complete adjuvant significantly increased levels of these parameters, whereas in drug treated and standard drug Diclofenac sodium treated groups, a marked decrease in the levels was observed. Arthritic Index was also found to be low in treated groups as compared to model control group. **Conclusion:** Methanolic extract of leaves of *Rivea hypocrateriformis* showed significant anti-arthritic activity against Freund's complete adjuvant induced arthritis in rats.

Key words: Arthritis, *Rivea hypocrateriformis*, Freund's Adjuvant, Diclofenac sodium.

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1. INTRODUCTION

Rheumatoid Arthritis (RA) is an autoimmune, systemic, inflammatory disease of unknown etiology predominantly affecting the synovial joints and periarticular tissue [1].It also affects other tissues and organs like skin, blood vessels, heart, lungs and muscles[2].

It is a chronic, progressive, systemic, inflammatory joint disease characterized by inflammation of synovial

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membrane (synovitis) and progressive destruction of cartilage and bone, resulting in pain, stiffness and restricted joint movement [3] which is responsible for significant deformity and disability[4]. Rheumatoid arthritis is characterized by infiltration of a variety of inflammatory cells into the joint. The synovial membrane becomes highly vascularized, synovial fibroblasts proliferate and inflammatory cells release various cytokines and growth factors into the joint. These agents cause synovial cells to release proteolytic enzymes resulting in destruction of bone and cartilage. Increase production of pro-inflammatory cytokines like IL-1, IL-6, TNF- and IL-18 also play an important role in pathogenesis of RA [5].

It is characterized by synovial hyperplasia, angiogenesis and mononuclear infiltration. It progresses in 3 stages. The first stage is swelling of synovial lining causing pain, warmth, stiffness, redness and swelling around the joint. Second stage is rapid division and growth of cells which causes the synovium to thicken. In the third stage, the inflamed cells release enzymes that digest bone and cartilage, leading to excessive pain and loss of movement [6].

The prevalence of RA worldwide varies between 0.3- 1% and is more in developed countries[7]. The prevalence of RA in Indian subcontinent is 1.5-2% of population[3]. Women are affected more often than men at a ratio of 3:1 [8].

The drugs commonly used for the treatment of RA include steroids, Non steroidal anti-inflammatory drugs (NSAIDs), Disease modifying anti-rheumatoid drugs (DMARDs) and immunosuppressive drugs. All these drugs besides being costly are associated with numerous side-effects, severe adverse reactions and toxicity [9]. Hence, the field of arthritis treatment research has progressed towards herbal therapies that are considered safe and effective in relieving chronic pain associated with arthritis[10]. In India, many Ayurvedic practitioners are using various plants for treatment of different types of arthritic conditions based on their traditional use. However, it is essential to investigate the rationality of their use in modern scientific terms [11]. Agents derived from plants that can modulate the expression of pro-inflammatory signals clearly have potential against arthritis. These include flavanoids, terpenes, quinones, catechins, alkaloids, anthocynins, anthoxanthins, all of which have anti-inflammatory effects [12].

Rivea hypocrateriformis belongs to family convolvulaceae. Leaf and young shoots are eaten as vegetables. Juice of leaf is used for skin diseases of hair and scalp. Juices of leaves along with babul twig and sugar is taken with cow's milk for relief from rheumatic pain [13, 14]. The alcoholic extract of whole plant of *Rivea hypocrateriformis* has been reported to exhibit anti-implantation activity in female albino rats [15, 16]. Although the leaves of *Rivea hypocrateriformis* have been used for long time in the treatment of inflammation and pain, anti-arthritic activity of leaves is not supported by any scientific base. So, the present study was designed to evaluate the anti-arthritic activity of *Rivea hypocrateriformis* leaves.

2. MATERIALS AND METHODS

Plant material:
The fresh aerial parts of *Rivea hypocrateriformis* were collected in the month of December within the campus of New Vallabh Vidyanagar, Dist. Anand, Gujarat. The plant was identified and authenticated at the Department of Biosciences, Sardar Patel University, Vallabh Vidyanagar. A voucher specimen of the plant (**RRK/IE-02/15/ARGH-11)** has been deposited in the Herbarium of Department of Pharmacognosy, A.R.College of Pharmacy & G.H.Patel Institute of Pharmacy, Vallabh Vidyanagar, Anand, India.

Preparation of methanolic extract:
The aerial parts of *Rivea hypocrateriformis* were shade dried at room temperature and pulverized in grinder and stored in air tight container for further use. 500 g of dried powdered drug was extracted with required quantity of methanol for eight hours, filtered and named as Methanolic extract of *Rivea hypocrateriformis* (**MRH**). The methanolic extract was evaporated to dryness under reduced pressure at low temperature (percent yield-7.07).

Phytochemical analysis: [17]
The preliminary phytochemical screening of the Methanolic extract of *Rivea hypocrateriformis* (**MRH**) was carried out as per the standard procedures, to detect the different constituents present in it. The extract was tested for phytosterols, alkaloids, saponins, flavanoides, phenolic substances , coumarin glycosides, fixed oil, free amino acids and carbohydrates.

Drugs and Reagents:
Carageenan –Sigma Aldrich, Mumbai
Diclofenac sodium- Crystal Pharma, Mumbai

Selection of animals:
Wistar rats (150- 200g) of either sex were used for the study. The animals were housed under standard conditions i.e. 26 ± 2^0C temperature and relative humidity 45-55%, maintained on 12 hr.light /dark cycle. The animals had free access to standard pellet diet and water *ad libitum*. The animals were acclimatized to the laboratory environment 1 hr. before the experiment. All experiments were conducted during the light period (8.00-16.00hrs.) The protocol **(No:PBRI/11/IAEC/PNS-29)** of the study was approved by Institutional Animal Ethics Committee of Pinnacle Biomedical Research Institute, Bhopal and animal study was performed at the same institute, as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Experimental Procedure:
24 rats were divided into four groups with 6 animals in each group**.**
Group I: Model control group, administered Complete Freund's adjuvant (CFA)

0.1ml; by sub-plantar injection .

Group II: Standard group, administered CFA 0.1ml+Diclofenac sodium-15mg/kg; ip, for 21 days.

Group III: Drug treated group (low dose), administered CFA 0.1ml + MRH- 250 mg/kg; p.o, for 21 days

Group IV: Drug treated group (high dose), administered CFA+ 0.1ml + MRH- 500 mg/kg; p.o, for 21 days

Anti-arthritic activity:

Complete Freund's adjuvant (CFA) induced arthritis in rats [18, 19, 20]

0.1 ml of CFA was injected into the sub plantar region of the left hind paw of all animals on day zero. CFA consists of 6mg *Mycobacterium butyricum* suspended in heavy paraffin oil by thorough grinding with mortar and pestle to give a concentration of 6mg/ml.

MRH 250 mg/kg; p.o., 500 mg/kg; p.o. and Diclofenac sodium at 20 mg/kg/alternate day; p.o were administered for 12 days from the day of CFA injection. Initial paw volume and initial body weight were recorded on the day of injection.

On day 4, the volume of the paw injected with CFA was measured again, to check development of primary lesion and the influence of therapeutic agents on this phase. The severity of induced adjuvant disease was assessed by measuring volume of non-injected paw and comparing it with that of injected paw.

Purposefully, from day 13 to 21, the animals were not dosed with the test compound or standard. On 21st day, blood was withdrawn from retro-orbital plexus of all rats of all the groups, various haematological and biochemical parameters were estimated [21, 22] .

Arthritic index:

All the animals were closely observed and scored for calculation of arthritic index as described in Table:1 The average scores for each group of drug treated animals were compared with that obtained for disease control animals.

Table 1: Scoring pattern for calculation of Arthritic Index

Organ	Indication	Score
Ears	Absence of nodules & redness	0
	Presence of nodules & redness	1
Nose	No swelling of connective tissue	0
	Intensive swelling of connective tissue	1
Tail	Absence of nodules	0
	Presence of nodules	1
Forepaws	Absence of inflammation	0
	Inflammation of at least 1 joint	1
Hind paws	Absence of inflammation	0
	Slight inflammation	1
	Moderate inflammation	2
	Marked inflammation	3

Body weight:

Body weight of each animal was taken on the day of administration of CFA, and then on completion of treatment period, on day 21.

Biochemical and hematological estimation:

From the blood samples collected, serum was separated and changes in levels of Biochemical parameters like SGOT, SGPT, ALP and haematological parameters like RBC, WBC, ESR and CRP were determined.

Paw edema:

Volume of both hind paws of all animals of all groups was recorded on the day of CFA injection and on 1, 4, 8, 14 and 21st day using mercury plethysmograph. The fifth day measurement is indicative of primary lesions and 14th day measurement is indicative of secondary lesions.

Statistical analysis:

Results are expressed as mean ± SEM. Statistical differences between the means of the various groups were analyzed using one way analysis of variance (ANOVA) followed by Dunnet's 't' test. Data were considered statistically significant at P value< 0.05.

3. RESULTS AND DISCUSSION

Phytochemical analysis:

The qualitative phytochemical analysis of methanolic extract of *Rivea hypocrateriformis* leaves showed the presence of alkaloids, flavanoids, coumarins, tannins and steroids.

Effect of MRH on arthritic index in CFA induced arthritic rats

Complete Freund's adjuvant induced arthritis model was used to induce arthritis in rats, since it is the best experimental model of Rheumatoid Arthritis which produces inflammation that is immunologically mediated [18]. It is the most widely used chronic test model in which clinical and pathological changes produced are comparable to those seen in human rheumatoid arthritis [23]. It produces chronic swelling in multiple joints with erosion of joint cartilage and bone destruction by increasing release of inflammatory mediators [24].

Mean arthritic index was found to be high in CFA induced arthritic rats. Arthritic index was significantly reduced on treatment with MRH (250 mg/kg, 500 mg/kg, p.o.) as compared to control group (p<0.01). Diclofenac sodium (20 mg/kg; p.o.) also reduced the arthritic index as compared to control group and the difference was found to be highly significant (p<0.001). **(Table 2)**

Table 2: Effect of MRH on arthritic index in Freund's adjuvant induced arthritis in Albino rats

Groups Dose/route	Arthritic index
Control	4.78± 0.89
Standard Diclofenac sodium (20 mg/kg; p.o.)	2.23 ± 1.21[##]
MRH 250mg/kg; p.o.	2.73 ±0.73[#]
MRH 500mg/kg; p.o.	2.37 ± 0.81[#]

Data are expressed as mean ± SEM. for n=6, Where [#] p<0.01, [##] p< 0.001 highly significant as compared to control by one way ANOVA followed by Dunnet's 't' test

Effect of MRH on changes in body weight on CFA induced arthritic rats

The body weight of animals injected with CFA (arthritic animals) was observed to be reduced. Treatment with MRH (250 mg/kg, 500 mg/kg p.o) for 21 days showed less decrease in body weight of animals as compared to control

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animals and the results obtained with high dose of MRH were comparable with those obtained with standard drug Diclofenac sodium (20 mg/kg; p.o.) treated group. Comparing the decrease in body weight in drug treated groups with CFA treated groups the difference was found to be highly significant (p<0.001). A report by Patil et al suggests that decrease in body weight during inflammation may be due to deficient absorption of nutrients through intestine [25]. The evident restoration of body weight of rats in Diclofenac sodium and MRH treated groups indicates normalization of the process of intestinal absorption of nutrients through the intestine. Thus changes in body weight in CFA and drug treated rats help to assess the progress of disease and response to anti-arthritic drugs [26]. **(Table.3)**

Table 3: Effect of MRH on changes in body weight in CFA induced arthritic rats

Group Drug treatment and Dose (mg/kg)	Percentage reduction in body weight
Control group CFA 0.1ml + Saline	18.87
Standard group CFA 0.1ml + Diclofenac sodium (20 mg/kg; p.o.)	10.08[#]
Drug Treatment (low dose) CFA 0.1ml + MRH (250mg/kg; p.o.)	12.34[#]
Drug Treatment (high dose) CFA 0.1ml + MRH (500mg/kg; p.o)	11.08[#]

Data are expressed as percentage. # p<0.001 highly significant when compared to control by one way ANOVA followed by Dunnet's 't' test.

Effect of MRH on changes in biochemical parameters in CFA induced arthritic rats

Injection of CFA increased levels of SGOT, SGPT and ALP in rats of control group. **Table 4** shows the changes in biochemical parameters in drug treated groups as compared to arthritic control group. Assessment of levels of SGOT, SGPT and ALP acts as an excellent tool to measure anti-arthritic activity of a drug. The levels of these enzymes are significantly increased in arthritic rats. Serum SGOT and SGPT have been reported to play a vital role in the formation of biologically active chemical mediators like bradykinins in inflammatory process. Also elevated levels of ALP in arthritic rats may be due to increase in liver and bone fraction indicating bone loss or erosion [27, 28]. Drug treated groups showed reduction in levels of these biochemical parameters signifying good anti-arthritic potential, comparable with standard drug Diclofenac Sodium and the difference in parameters was found to be highly significant (p<0.001).

Table 4: Effect of MRH on changes in biochemical parameters in CFA induced arthritic rats

Group Drug treatment and Dose (mg/kg)	Biochemical Parameters		
	SGOT (U/L)	SGPT (U/L)	ALP (U/L)
Control group CFA 0.1ml + Saline	119.83±6.4	94.83±8.58	55.33±7.73
Standard group CFA0.1ml+Diclofenac sodium (20 mg/kg; p.o.)	72.16±5.34[#]	56.16±5.94[#]	38.33±4.13[#]

| Drug Treatment (low dose) CFA 0.1ml + MRH (250mg/kg; p.o.). | 105.83±4.87 | 71.66±5.75[#] | 48.66±2.58 |
| Drug Treatment (high dose) CFA 0.1ml + MRH (500mg/kg; p.o.). | 98.16±7.05[#] | 63.83±3.65[#] | 41.83±4.79[#] |

Data are expressed as mean ± SEM. for n=6, Where # p< 0.001 highly significant compared to control; by one way ANOVA followed by Dunnet's 't' test

Effect of MRH on changes in haematological parameters in CFA induced arthritic rats

CFA also induced changes in various hematological parameters like WBC count, RBC count, ESR and CRP. Increase in ESR and WBC count is a common feature of inflammatory reactions, since inflammation is mediated by increase in production of lymphocytes and migration of leucocytes into inflamed area [3]. Thus the increased WBC count and ESR value in arthritic control group of rats was reduced in drug treated groups, indicating inhibition of migration of leucocytes which has beneficial effect in preserving the joints. Comparing the results of drug treated groups with the control group, the difference was found to be highly significant (p<0.001).

C reactive protein (CRP) is a hepatically derived marker of systemic inflammation. Its levels increase dramatically during inflammatory process occurring in the body due to increase in plasma concentration of IL-6 [29]. CFA increased CRP level in arthritic rats, which was significantly reduced after administration of MRH signifying its efficacy in treatment of arthritis.

Table 5: Effect of MRH on changes in haematological parameters in CFA induced arthritic rats

Groups Drug treatment and Dose (mg/kg)	Haematological Parameters				
	RBC (millions/mm3)	WBC (thousands/mm3)	ESR (mm/hr)	PLATELETS (lakhs/mm3)	CRP (mg/l)
Control group CFA0.1ml + Saline	7.88±0.23	12.96±1.63	13.16±1.47	4.96±0.109	418.66±5.95
Standard group CFA0.1ml + Diclofenac sodium (20 mg/kg; p.o.)	9.46±0.21[#]	6.58±1.13[#]	10.5±1.87[#]	4.13±0.057[#]	242.16±6.21[#]
Drug Treatment (low dose) CFA 0.1ml + MRH (250mg/kg; p.o).	8.1±0.25	10.1±1.87[#]	12.16±1.38	4.65±0.047	325.16±6.17[#]
Drug Treatment (high dose) CFA 0.1ml + MRH (500mg/kg; p.o.)	8.36±0.21[#]	8.83±1.41[#]	11.5±1.22[#]	4.32±0.069	277.5±6.77[#]

Data are expressed as mean ± SEM. for n=6, Where # p< 0.001 highly significant compared to control; by one way ANOVA followed by Dunnet's 't' test

Effect of MRH on paw edema in CFA induced arthritic rats

Administration of CFA into sub plantar region of left hind paw of rats resulted in significant increase in paw volume

over the period of 21 days. Treatment with MRH (250 mg/kg, 500 mg/kg p.o) and Diclofenac sodium (20 mg/kg; p.o) for 21 days, reduced the paw volume as compared to control group probably by suppressing the release of inflammatory mediators and the difference was found to be highly significant (p<0.001).

Table 6: Effect of MRH on paw edema in CFA induced arthritic rats

Groups Drug treatment and Dose (mg/kg)	DAY 1	DAY 21	% Inhibition of paw volume
Control group CFA 0.1ml+ Saline	1.222±0.07	0.987±0.079	_
Standard group CFA 0.1ml+ Diclofenac sodium (20 mg/kg; p.o.)	1.176±0.08	0.240±0.047#	75.75
Drug Treatment (low dose) CFA 0.1ml + MRH (250mg/kg; p.o.)	1.069±0.05#	0.351±0.043#	64.56
Drug Treatment (high dose) CFA 0.1ml+MRH (500mg/kg; p.o)	1.040±0.06#	0.264±0.032#	73.33

Data are expressed as mean ± S.E.M (n=6) #p< 0.001 highly significant as compared to control by one way ANOVA followed by Dunnet's 't' test.

4. CONCLUSION

Thus the results of the present study lead to the conclusion that MRH possesses good anti-arthritic activity. Though its actual mechanism of suppressing inflammation is not known, it can be correlated with presence of phytoconstituents like alkaloids, flavanoids, steroids and tannins. Other researchers have also reported the effectiveness of flavanoids and alkaloids in suppressing inflammation associated with arthritis [3, 30, 8].

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