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Research Article

Effect of Biochemical Parameters in Cetylmyristeolate Treatment Against Freund's Adjuvant Induced Arthritis Model

*Kayalvizhi M.K., Vanitha Samuel, P. Nirmala

Division of Pharmacology, RMMC & H, Annamalai University, Annamalai Nagar, Chidambaram, Tamilnadu.

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ABSTRACT

The current research was to evaluate the effect of Cetylmyristeolate (CMO) in Freund's complete adjuvant induced arthritis in rats by analyzing its biochemical parameters. The experimental study was performed on Wister rats, Freund's complete adjuvant was injected to induce arthritis in tibiotarsal joint (IA), CMO-500mg; 1g and CMO 100mg + MSM 200mg + glucosamine 500mg /day for 21 days were given. Blood was collected by intraocular puncture on days 1,7,14 and 21and ESR (mm), serum CRP (mg/l) and Rheumatoid factor (IU/ml) were studied, the results showed that marked reduction of arthritic and inflammatory effect by CMO- 500mg/day and 1gm/day as well as the combination of 100mg/day + glucosamine 500mg and MSM 200mg /day.

Keywords: Arthritis, Rheumatoid factor, C – reactive protein, Erythrocyte sedimentation rate.

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disease a chronic systemic inflammatory disorder affects flexible (synovial) joints results in disabling and painful condition. Treatments are pharmacological and non-pharmacological. Non-pharmacological treatment includes physical therapy, occupational therapy and nutritional therapy but these don't stop the progression of joint destruction [(1)]. RA affects a disproportionate number of young- to middle aged adults, but its impact on older adults may be greater, given an increased incidence of concomitant disease for that population [(2)]. Non steroidal anti-inflammatory drugs have been the main stay of treatment in Rheumatoid arthritis conditions. But their wide spread use is associated with significant toxic effects especially on the gastrointestinal tract, liver and kidneys. The discovery of cetylmyristoleate an ester of fatty acid natural substances shown to be a less toxic and more effective means of achieving relief. Freund's complete adjuvant induced arthritis in rats is an excellent experimental model and is well documented [(3, 4)]. There are innumerable drugs for manifestations of the disease yet; there remains an urgent need for finding pharmacological therapies for arthritis which are effective, relatively safe and least toxic. By keeping the above factors it was aimed to treat Freund's complete adjuvant induced arthritis in rats through a natural substance called Cetylmyristeolate and compare its efficacy alone and in combination with other conventionally used preparations [(5, 6)].

MATERIALS AND METHODS

Animals: The institutional Animal ethical committee, RMMC & H, (proposal No. 549, dated 20.02.2008), Annamalai University, Annamalai Nagar, India approved the experimental design. Albino Wistar rats of 180-200g both sex were used for the study. Animals were housed in well ventilated room (temperature 23 ± 2°C, humidity 65 - 70% and 12h light / dark cycle at central animal house, Rajah Muthaih Medical College and Hospital, Annamalai University. Animals were fed with standard pellet diet and water *ad libitum*. All studies were conducted in accordance with committee for the purpose of control and supervision on experiments animals (CPCSEA) norms and the national institute of Health guidelines "Guide for the care and use of Laboratory Animals" [(7)].

Induction of Chronic Inflammation –by Freund's complete adjuvant induced arthritis model in rats: Rats were divided into six groups (n = 6). Experimental Arthritis was induced by injecting 0.1ml of Freund's complete Adjuvant into the tibiotarsal joint of left hind paw intrarticularly groups II to VI. Group I (normal control) treated with distilled water for 21 days per oral; Group II (Freund's complete adjuvant) 0.5 ml of FCA on first day and further distilled water for 21 days. Group III (Dexamethasone) 0.5 ml of on first day and further Dexa (0.1mg /kg body weight p.o) for 21 days; Group IV (test 1) drug CMO 0.5 ml of FCA on first day further CMO (500mg/day p.o) for 21 days; Group V (test 2) drug CMO 0.5 ml of FCA on first day and further CMO(1g/day p.o) for 21 days; Group VI (test 3) drug CMO + MSM + Glucosamine 0.5 ml of FCA on first day and further CMO(100mg) + MSM(200mg)+ glucosamine (500mg) /day for 21 days/p.o. The edema formation and percentage inhibition of paw volume was calculated on days 1,7,14 and 21 for all the groups as mentioned in our earlier publication (6). Blood was collected by intraocular puncture on 1,7,14 and 21 days.

Serum biochemical parameters:

ESR[(8)]: In the Westergren method rat blood 2ml was mixed with 0.5 ml of sodium citrate. The blood is drawn into a

*Author for correspondence: E-mail: drkayalsiva@rediffmail.com

Table 1: Serum biochemical parameters – ESR (mm)

Groups	Days 1	Days 7	Days 14	Days 21
Group I	8	5	5	4
Group I	29	37	40	27
Group II	6	7	9	17
Group IV	18	30	21	15
Group V	17	29	23	15
Group VI	15	25	10	20

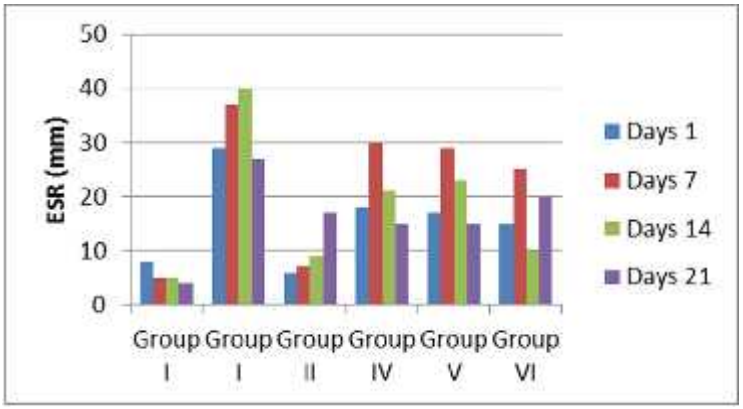

Figure 1: Serum biochemical parameters – ESR (mm)

Table 2: Serum biochemical parameters – CRP (mg/l)

Groups	Days 1	Days 7	Days 14	Days 21
Group I	6.6	8.2	9.2	6.2
Group II	18.0	20	24.4	23.6
Group III	8.5	13.5	11.2	7.2
Group IV	8.0	8.8	9.4	10.4
Group V	8.5	9.0	9.8	7.6
Group VI	8.5	12.4	8.0	6.4

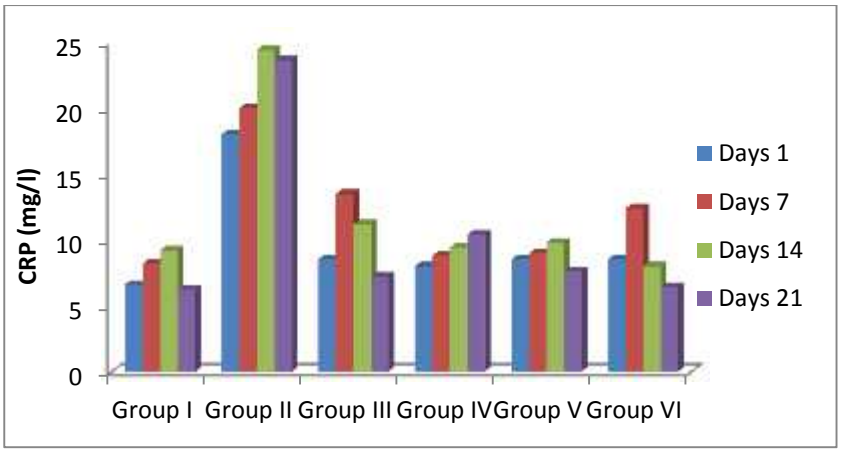

Figure 2: Serum biochemical parameters- CRP (mg/l)

Table3: Serum biochemical parameters – RA Factor (Iu/ml)

Groups	Days 1	Days 7	Days 14	Days 21
Group I	18.2	22.4	16.4	15.8
Group II	150	200	216	186
Group III	130	188	50	14.4
Group IV	135	162	48.6	15.8
Group V	140	129.9	70.8	15.4
Group VI	120	102.2	32.3	12.8

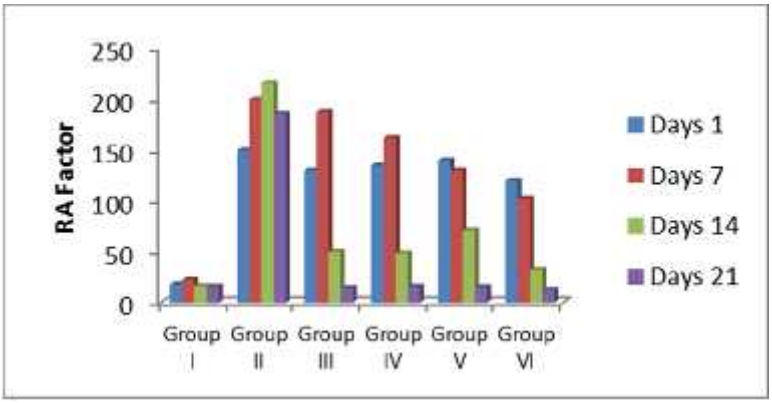

Figure 3: Serum biochemical parameters – RA Factor (Iu/ml)

Westergren-Katz tube to the 200 mm mark. The tube is placed in a rack in a strictly vertical position for 1 hr at room temperature, at which time the distance from the lowest point of the surface, meniscus to the upper limit of the red cell sediment is measured. The distance of fall of erythrocytes, expressed as millimeters in 1 hr, is the ESR.

*Figure 3: Serum biochemical parameters – RA Factor (Iu/ml)*CRP [9]: Serum was separated and CRP concentrations were determined by turbidimetric immunoassay (TIA) using liquid phase immunoprecipitation reaction with polyclonal antibodies obtained by rabbit immunisation with rat CRP (Protiline, BioMerieux). The formation of insoluble antigen-antibody complexes was monitored at 340 nm by the increase in turbidity, which is proportional to the concentration of CRP in the sample.

Rheumatoid factor **[10]:** The technique is based on the action of 0.1 M, 2-mercaptoethanol mixed directly with the serum. After 2 h of incubation determinations of rat anti-IgG were performed by latex agglutination test by Comparison with data obtained by using the conventional method, sequential 24 h treatment with 0.1 M 2-mercaptoethanol and 0.01 M iodoacetamide, shows similar results for both methods.

RESULTS AND DISCUSSION

The ESR values in group II were persistently high when compared to normal control. There was a significant reduction observed in the values in group III while the values in groups V and VI did not show much change (Vide Table. 1, Fig.1).The serological estimation of C – reactive protein, a more reliable serological marker of acute inflammation showed a statistically significant lowering of serum CRP values in Group IV to VI with values almost equal to normal in group VI. These values were comparable to both the normal animals in group I and the standard (*i.e*) Dexamethasone treated group III (vide Table.2. Fig.2). Rheumatoid factor estimation showed a significant rise in values in all except group I almost from day 1 to day 7 after which there was a reduction in the serological values in groups IV to VI which was comparable to the reduction in the standard group III (Vide Table.3, Fig.3).CMO at both doses showed a statistically significant reduction in biochemical values.

It was persumed that CMO a medium chain fatty acid is not an analgesic perse but reduces inflammation by normalizing hyper immune responses. CMO stimulates the production of prostaglandin series 1 and 3 which were known to suppress inflammation (anti-inflammatory) unlike the series 6 prostaglandins were known to enhance inflammation (pro-inflammatory), this probably accounts for its anti- inflammatory action. Though the biochemical events in the various phases were not clearly elucidated, many factors implicated were calcium chemotoxin, leukocyte promoting factors, & complement factors which save as regulators of phagocytosis **[11]**. CMO facilitates cartilage building & also serves as a surfactant/ lubricant for the damaged joints. Several studies have documented the actions of Glucosamine, a mucopolysaccride which

prevents inflammation by decreasing the generation of superoxide radical [12,13].

CONCLUSION

The present research concludes that CMO, a acetylated fatty acid ester produced significant effect in reducing adjuvant induced arthritis. Though the actual mechanism of action of CMO as an anti-inflammatory agent is still hypothetical, its effect in reducing the signs of inflammation and improving joint mobility with the least possible adverse effects makes it a remarkable drug in the treatment of degenerative conditions like OA & auto immune diseases like RA.

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