Effect on the biochemical and hematological parameters on *Rasa Mezhugu* in Freund’s adjuvant-induced arthritis rats

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ABSTRACT

Rheumatoid Arthritis (RA) is a chronic, destructive inflammatory poly-articular joint and systemic autoimmune disease. It typically results in warm, swollen, and painful joints, stiffness often worsen following rest. The goal of treatment is to reduce pain, decrease inflammation, and improve a person's overall functioning. In spite of tremendous development in the field of synthetic drugs during recent years, the side effects could not be avoided. Hence the current research focus is to develop less toxic drugs as early as feasible in the disease process. A 90 day old Wistar male rat was used for the research and was divided in to four groups treated with Saline, *Palm Jaggary*, *Rasa Mezhugu* and Indomethacin. Evaluation of anti-arthritic potency, by body weight, biochemical and hematological analysis were analyzed. *Rasa Mezhugu* treatment rats have significantly reduces the Arthritis effect induced by complete Freund’s adjuvant (CFA)-induced arthritic rat.

Keywords: *Rasa Mezhugu*, *Palm Jaggary*, Indomethacin, biochemical analysis and Rheumatoid arthritis.

INTRODUCTION

Rheumatoid arthritis (RA) is the most common type of autoimmune arthritis affects the wrist and characterized by joint swelling, synovial membrane inflammation and cartilage destruction. RA, early treatment can control joint pain and swelling, and lessen joint damage. RA is a systemic inflammatory disorder that affects approximately 1% of the population worldwide. The severity and disease progression of RA are governed by multiple factors including immune, genetic and environmental factors (1,2). Multiple components of immunity and inflammation play a role in the onset and progression of the disease, including T and B lymphocytes, neutrophils, monocytes and the vascular endothelium. It is strongly associated with the inherited tissue type major histocompatibility complex (MHC) antigen HLA-DRB1 and the genes PTPN22 and PADI4—hence family history is an important risk factor. Vitamin D deficiency is more common in people with rheumatoid arthritis than in the general population. The various phases of progression of RA are Initiation phase, due to non-specific inflammation; Amplification phase, due to T cell activation and chronic inflammatory phase with tissue injury, due to cytokines IL–1, TNF-alpha and IL–6 (3). The herbal medicines based on preliminary promising results Boswellic acid, Curcumin, Devil's claw, Euonymus alatus, and Thunder god vine (*Tripterygium wilfordii*). Increasing intake of fiber from fruits, vegetables and whole grains may also help reduce inflammation. Studies show that adding fiber to the diet results in lower levels of C-reactive protein (CRP) in the blood; CRP is an indicator of inflammation. In the same way that a nonsteroidal anti-inflammatory drug (NSAID) such as ibuprofen or aspirin can – it contains a compound called oleocanthal that blocks the enzymes that cause inflammation(4).

Mechanical allodynia, thermal hyperalgesia and pain on joint movement (joint hyperalgesia), which are prominent features in arthritic pain. The complete Freund's adjuvant (CFA)-induced arthritic rat model has extensively served as a laboratory model in the study of arthritic pain. Important criteria in selection of this model include capacity to predict efficacy of agents in humans, ease of performing the model, reproducibility of data, reasonable duration of test period and similar pathology and/or pathogenesis to that of human disease. Rat arthritic arthritis is an experimental
model of polyarthritis which has been widely used for preclinical testing of numerous anti-arthritic agents which are either under preclinical or clinical investigation or are currently used as therapeutics in this disease(5).

The current research focus is to develop less toxic drugs as early as feasible in the disease process. In spite of tremendous development in the field of synthetic drugs during recent years, the side effects could not be avoided. Therefore, efficacy of various plants against inflammation and arthritis has been explored. Now it is a growing concern allover for the development of new safe, potent, less toxic antiarthritic drug. Hence, there is a need to explore for more naturally available alternatives, so that their therapeutic values can be assessed and expanded.

MATERIALS AND METHODS

Animals: A 90 day old Wistar rat bred and raised in the Animal Facility of the Department of Pharmacology, Vel’s college of Pharmacy were used in this study. They were maintained under constant automatically control 12 h/12 h light/dark cycle (lights on from 07.00 a.m. to 07.00 p.m.) and environmental temperature (23±2°C). Rat chow and tap water were provided ad libitum in standard propylene cages. Cage cleaning consisted of daily change of sawdust bedding. At the end of the study, animals were sacrificed with an overdose of Chloral hydrate. Due to the painful condition imposed on the animals, the number of subjects used was restricted to the minimum that allowed reliable statistical analysis of the results. All procedures were submitted to and approved by the Ethics Committee and followed the recommendations of the Research and Ethics Committee. Each group was composed of 6 animals (6, 7).

Drug material and stock solution: The semi-solid form of Rasa Mezhugu was mixed uniformly in Palm jaggary (100mgw/v) in saline solution to achieve 100mg/ml as main stock solution and used in this study. All solutions were daily prepared and stirred until the residues were completely dissolved. The volume administered was 2ml/kg of body weight. Control animals received the same volume of Palm jaggary (100mgw/v) in saline.

Evaluation of anti-arthritic potency: Freud’s adjuvant induced arthritis model was used to assess the anti-arthritic activity in albino rats. Following anesthesia with diethyl ether, 0.1ml of Freund’s adjuvant (complete fraction of Mycobacterium butyricum suspended in mineral oil; was injected in the sub-plantar tissue of the right posterior paw and 100% of the animals developed arthritis. Everyday animals were carefully and thoroughly inspected, by examining the affected paw and the animal’s general status. Evaluation of the anti-arthritic effects of Rasa Mezhugu was performed by monitoring the edema in the right paw. In control animals, sub-plantar injection of Freud’s adjuvant produced a local edema after a few hours with a progressive increase reaching its maximum in the eighth day after inoculation. On day 0, all animals were subjected to behavioral test and assessment of body weight and right paw’s measurements, followed by the injection of Freud’s adjuvant in the right paw.

On the 7th day after adjuvant administration, animals were randomly distributed into four groups: Group I received the vehicle saline only. Group II received the vehicle (2 ml/kg body weight, Palm jaggary (100mgw/v) in saline) and served as arthritic control and group III received Rasa Mezhugu with Palm jaggary at a dose of 500mg/kg body weight. Group –IV animals were treated with Indomethacin (10mg/kg) and then again all those animals were subjected to behavioral test and assessment of body weight and paw’s edema measurements. The test solution was administered daily and testing application was done on Days 8, 13, 18, 23 and 28 after injection of Freund’s adjuvant. Paw volume was measured on 4th, 8th, 14th and 21st day with the help of Plethysmometer for both the ipsilateral (injected paw) and the contralateral paw (non-injected paw) before intraplantar injection of CFA (day 0) and every other day (8, 9).

Body weight, Biochemical and Hematological analysis: The changes in body weight were recorded daily. Blood sample was withdrawn through retro-orbital vein puncture of all groups by anaesthetizing the animals and for biochemical and hematological parameters. Estimation of RBC and WBC count were analyzed in an improved Neubauer chamber. ESR was estimated by the method of westergren technique. The biochemical marker enzymes such as ALT, AST, Total protein levels and CRP were analyzed in Secomam semi auto analyzer. The results were expressed as mean ± SEM; differences in mean were estimated by means of ANOVA followed by “Dunnet’s post hoc” test. All the data are presented as mean ± SEM and p<0.05 was considered as significant (10-13).

Table 1: Effect of Rasa Mezhugu on body weight in arthritic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose</th>
<th>0 day</th>
<th>8th Day</th>
<th>13th Day</th>
<th>18th day</th>
<th>25th day</th>
<th>28th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Saline</td>
<td>2ml/kg</td>
<td>164±2.0</td>
<td>169±2.0</td>
<td>174±2.2</td>
<td>178±1.8</td>
<td>180±2.2</td>
<td>182±2.1</td>
</tr>
<tr>
<td>Control (RA)</td>
<td>Palm Jaggary</td>
<td>2ml/kg</td>
<td>169±2.2</td>
<td>173±1.9</td>
<td>176±2.7</td>
<td>174±2.1</td>
<td>176±2.2</td>
<td>178±3.5</td>
</tr>
<tr>
<td>Test</td>
<td>Rasa Mezhugu</td>
<td>500mg/kg</td>
<td>168±2.0</td>
<td>171±1.8</td>
<td>173±2.5</td>
<td>173±2.0</td>
<td>175±2.4</td>
<td>174±2.6</td>
</tr>
<tr>
<td>Standard</td>
<td>Indomethacin</td>
<td>10mg/kg</td>
<td>165±1.8</td>
<td>167±1.5</td>
<td>166±2.8</td>
<td>168±1.9</td>
<td>172±2.6</td>
<td>178±2.8</td>
</tr>
</tbody>
</table>

Values are expressed as (Mean ± SEM) N = 6; One way ANOVA followed by Dunnet`s test, p<0.01 vs 0day

Table 2: Effect of Rasa Mezhugu on Biochemical parameters in CFA induced arthritic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose</th>
<th>ALT (IU)</th>
<th>AST (IU)</th>
<th>TP (g/dl)</th>
<th>CRP (μg/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Saline</td>
<td>2ml/kg</td>
<td>33.15±0.77</td>
<td>101.71±6.31</td>
<td>11.10±0.98</td>
<td>168.31±4.2</td>
</tr>
<tr>
<td>Control (RA)</td>
<td>Palm Jaggary</td>
<td>2ml/kg</td>
<td>46.64±0.92</td>
<td>140.22±10.82</td>
<td>14.26±1.12</td>
<td>398.20±8.7</td>
</tr>
<tr>
<td>Test</td>
<td>Rasa Mezhugu</td>
<td>500mg/kg</td>
<td>28.36±0.51</td>
<td>88.14±7.22</td>
<td>10.34±0.74</td>
<td>264.00±6.5b</td>
</tr>
<tr>
<td>Standard</td>
<td>Indomethacin</td>
<td>10mg/kg</td>
<td>22.08±0.48</td>
<td>57.90±3.67</td>
<td>8.82±0.42</td>
<td>201.19±4.6b</td>
</tr>
</tbody>
</table>

Values are expressed as (Mean ± SEM) N = 6; One way ANOVA followed by Dunnet`s test, bP<0.01 vs Normal, *P<0.05, **P <0.01 vs arthritic control

Table 3: Effect of Rasa Mezhugu on hematological parameters in arthritic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose</th>
<th>TWBC count (Cells/cu.mm)</th>
<th>RBC count (millions/cu.mm)</th>
<th>Hb (gm %)</th>
<th>ESR (mm/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Saline</td>
<td>2ml/kg</td>
<td>7.68±0.06</td>
<td>5.34±0.14</td>
<td>14.22±0.10</td>
<td>2.18±0.16</td>
</tr>
<tr>
<td>Control (RA)</td>
<td>Palm Jaggary</td>
<td>2ml/kg</td>
<td>7.54±0.08</td>
<td>5.12±0.16</td>
<td>12.51±0.26</td>
<td>4.06±0.11</td>
</tr>
<tr>
<td>Test</td>
<td>Rasa Mezhugu</td>
<td>500mg/kg</td>
<td>7.72±0.04</td>
<td>5.49±0.19</td>
<td>14.10±0.12</td>
<td>3.27±0.16</td>
</tr>
<tr>
<td>Standard</td>
<td>Indomethacin</td>
<td>10mg/kg</td>
<td>7.60±0.08</td>
<td>5.43±0.11</td>
<td>14.63±0.15</td>
<td>3.01±0.13</td>
</tr>
</tbody>
</table>

Values are expressed as (Mean ± SEM) N = 6; One way ANOVA followed by Dunnet`s test, bP<0.01 vs Normal, *P<0.05, **P <0.01 vs arthritic control

Fig 1: Effect of Rasa Mezhugu on biochemical parameters in arthritic rats

Fig 2: Mean body weight changes
Effect on body weight & hematological parameters: There is a significant increase in body weight Rasa Mezhugu treated group from 0 day 28th day. In the studies, there is an increased ESR level, which is a common diagnostic feature of patient with chronic arthritis. Increase in the erythrocyte sedimentation rate is an indication of active but obscure disease process, which elevate in response to stress, inflammation and cell necrosis. In arthritic condition there is mild to moderate increase in the WBC count which plays a major role in body defense mechanism. WBC count increase is may be due to the release of interleukins, responsible for production of both granulocytes and macrophages colony stimulating factor. Treatments with the Rasa Mezhugu 500 mg/kg significantly decrease the ESR and the WBC count indicates the significant recovery from the arthritic progress. Similarly the Hb level was significantly increased in drug treated group compared to control (14, 15).

Effect on biochemical estimation: The challenge with CFA (1%, 0.1ml) showed significant (p<0.05) elevation of the serum SGOT and SGPT level and decrease in the TP level, whereas the effect on ALP was insignificant in arthritis control as compared to normal control. The treatment with Rasa Mezhugu 500 mg/kg showed significant (p<0.05) prevention of the elevation of the serum SGOT & the decrease in the TP level as compared to arthritis control, whereas the effect on SGPT and ALP was insignificant. Indomethacin showed significant (p<0.01) prevention of the elevation of the serum SGOT and SGPT level and the decrease in the TP level as compared to arthritis control, and the effect on ALP was significant. CFA induced arthritis involves highly significant increase paws thickness, decrease in serum cortisol, significant increase in C-Reactive proteins. The acute phase proteins in ESR and C-Reactive Proteins (CRP) share the property of showing elevations in the concentration in response to stress or inflammations like infection, injury, and surgery in tissue necrosis (16, 17). Results of ESR and C-Reactive Proteins substantial and correlate with the above statement by showing significant decrease in serum after treatment with Rasa Mezhugu 500 mg/kg. p.o.

Assessment of the serum levels of AST, ALT and ALP provides an excellent and simple tool to measure the anti-arthritic activity of the target drug. The activities of amino transferases and ALP increases significantly in arthritic rats, since these are good indices of liver and kidney impairment which is also considered a feature of adjuvant arthritis. Serum AST and ALT has been reported to play a vital role in the formation of biologically active chemical mediators such as bradykinins in inflammatory process (18-20).

Elevated levels of serum ALP in adjuvant induced arthritic rats can be due to increase in the liver and bone fraction or due to an increase of both isoenzymes. This in turn implicates a localized bone loss in the form of bone erosion and peri-articular osteopenia, as the enzyme is released into circulation in the course of bone formation and resorption. It was reported that adjuvant administration in rats, immunologically alters the hepatic biochemistry. Most reports suggested an increase of the baseline serum ALP level in RA, although underlying mechanism remains uncertain. Also adjuvant treated rat showed significantly higher serum biochemistry markers (AST, ALT and ALP) than normal rats.

CONCLUSION

In the present study, the challenge with CFA (1%, 0.1 ml) was significantly (p<0.01) elevated the serum AST and ALT estimated as serum SGOT and SGPT level and decreased the TP level, whereas the effect on ALP was insignificant in arthritis control as compared to normal control. This was suggested that the development of inflammation in CFA induced arthritis may be related to the biochemical changes in hepatic enzymes. The administration of Rasa Mezhugu 500 mg/kg was significantly (p<0.05) reversed the elevated amino transferases. This effect may be related to their anti-inflammatory activity. The decreased enzyme levels on Rasa Mezhugu treatment emphasize on the decreased bone loss and organo protective roles of Rasa Mezhugu in adjuvant induced arthritis rats. Thus, these findings indicated the inhibition of lymphocytes and decreased immunological response that may be responsible for the anti-arthritic potential of Rasa Mezhugu. The initial reduction of edema and soft tissue thickening at the depot site could probably be due to the effect of the adjuvant, whereas the late occurring disseminated arthritis and flare in the injected foot were presumably immunological events.
REFERENCES