Screening of Anti-Arthritic, Anti-Inflammatory and Analgesic Activity of a Polyherbal Formulation

S. Meera, N.S. Kumar and V.S.S. Guptatyam
Visveswarapura Institute of Pharmaceutical Sciences, 22nd Main,
24th Cross, Banashankari 2nd Stage, Bangalore 560 070, India

Abstract: Anti-arthritic, anti-inflammatory and analgesic activity of Arthosansar (AS), a polyherbal formulation was evaluated and validated in various animal models. Arthritis was induced by Complete Freund's Adjuvant (CFA) injection in metatarsal footpad of Sprague-Dawley rats. Degree of inflammation was evaluated by hind paw swelling and body weight, estimation of AST, ALT and TP supported by histopathology of knee joint. AS reduced hind paw swelling, body weight, AST, ALT and TP. Histopathology revealed significant reduction in mononuclear infiltration, pannus formation and bone erosion. AS decreased the paw volume in carrageenan treated rats. As shows moderate central and peripheral analgesic activities in hot plate method and acetic acid induced writhing method in mice.

Key words: Arthosansar, complete Freund's adjuvant, AST, ALT, TP, pannus, analgesic

INTRODUCTION

Rheumatoid Arthritis (RA) is a chronic, destructive inflammatory polyarticular joint and systemic autoimmune disease of unknown cause (Hong-Kei et al., 2007). The prevalence of RA is consistent worldwide affecting, about 0.5-1.0% of the population. It usually occurs in people between 25 and 55 year of age. Women are affected more often than men at ratio of 3 to 1 (Maya et al., 2002). It is characterized by synovial hyperplasia, angiogenesis and mononuclear infiltration. RA progresses in three stages. The first stage is the swelling of the synovial lining, causing pain, warmth, stiffness, redness and swelling around the joint. Second is the rapid division and growth of cells, or pannus, which causes the synovium to thicken. In the third stage, the inflamed cells release enzymes that may digest bone and cartilage, often causing the involved joint to lose its shape and alignment, more pain and loss of movement (Atsushi et al., 2005). As the result of the inherent problems associated with current non-steroidal as well as steroidal anti-inflammatory agents, there is a continuous search especially from natural source. Arthosansar (AS), is a polyherbal formulation containing extracts of medicinal plants viz. Guggulu, Sallaki, Rasna, Eranda, Shunti, Shilajit and Aswagandha. These constituents are used in folk medicine for the treatment of inflammation and pain associated with rheumatoid arthritis, osteoarthritis, gout arthritis, sciatica, lumbar spondylitis, cervical spondylitis, neuromuscular disorders and rheumatic pains. The present study was undertaken to evaluate and to validate the anti-arthritic, anti-inflammatory and analgesic activity of AS.

MATERIALS AND METHODS

Drugs and chemicals: Poly Herbal Formulation (PHF), AS was a gift from Pradhan Herbal Company, Bangalore. The dry powder of polyherbal formulation was reconstituted using 0.5% w/v Sodium Carboxy Methyl Cellulose (SCMC) to get 1 mg ml-1 of Arthosansar. The suspension was freshly prepared before use. ALT, AST and TP kit from RMS Ltd., Baddi, Freund's adjuvant emulsion from Dlfo Lab, USA, Pethidine Sulphate from AstraZenica, Bangalore, Carrageenan from Sigma Labs and all other chemicals, reagents used were of analytical grade.

Animals: SD rats weighing 140±10 g of either sex and Swiss albino mice of either sex weighing 22±3 g, procured from NIMHANS, Bangalore and Veterinary College, Bangalore were used in this study. The animals were procured at least 2 weeks prior to the study and maintained in institutional animal house, (registration No. 152/1999/CPCSEA) so, that animals could adapt to the new environment. The Institutional Animal Ethics Committee's permission was obtained before starting the experiments on animals. The studies were conducted from August 2007 to March 2008.

Corresponding Author: S. Meera, Visveswarapura Institute of Pharmaceutical Sciences, 22nd Main, 24th Cross, Banashankari 2nd Stage, Bangalore 560 070, India Tel: 91-8026711651 Fax: 91-8026711851
**Grouping and treatment of experimental animals:** For Adjuvant induced arthritis model, Female Sprague-Dawley rats weighing 130-150 g were divided into five groups of six animals each. Control (Group 1) animals were administered the vehicle, Group 2 (Arthritic control) animals were administered the vehicle and CFA 0.1 mL to sub planter region of hind paw, Group 3 and Group 4 animals were administered the PHF 270 mg kg⁻¹, 540 mg kg⁻¹ and reference standard indomethacin, 5 mg kg⁻¹ p.o., respectively (Chamundeeswari et al., 2005). The lower and higher dose of polyherbal formulation was calculated according to daily human dose using conversion factor based on body surface area.

For Carageenan induced hind paw edema Albino Wistar rats weighing between 150-200 g were divided into four groups of six animals each; Control (group 1) animals were administered saline, Group 2 animals were administered Indomethacin (10 mg kg⁻¹), Group 3 animals were administered the polyherbal formulation lower dose, 270 mg kg⁻¹, Group 4 animals were administered the polyherbal formulation higher dose, 540 mg kg⁻¹.

For Eddy’s hot plate Swiss albino mice of either sex were divided into four groups of six animals each. Control (Group 1) animals were administered the vehicle, Group 2-4 animals were administered the AS 400 mg kg⁻¹, 800 mg kg⁻¹ and Pethidine sulfate 5 mg kg⁻¹ i.p., respectively.

For acetic acid induced writhing, Swiss albino mice of either sex were divided into four groups of six animals each. Control (Group 1) animals were administered the vehicle, Group 2-4 animals were administered the AS 400 mg kg⁻¹, 800 mg kg⁻¹ and indomethacin 10 mg kg⁻¹ p.o., respectively. The doses of polyherbal formulation are based on the daily human dose.

**Experimental**

**Adjuvant induced arthritis model:** Arthritis was induced by a 0.1 mL injection of complete Freund’s adjuvant emulsion (CFA) into the sub-planter surface of right hind paw (Mi-jung et al., 2006). Drugs was administered orally once a day, from the day of injection of CFA and continued up to 14th post CFA challenge day. The change in the inflammatory reaction was measured using mercury plethysmograph on 0, 4, 7, 14 and 21 day from the day of CFA injection. The animals were weighed, using digital weighing balance, on 0, 4, 7, 14 and 21 day from the day of CFA injection (Abdul-Shakoor et al., 2007) At the end of 21st day rats were anaesthetised with diethyl ether. Blood was withdrawn by puncture of retro orbital plexus, centrifuged (Remi) and serum ALT, AST and TP estimated (Colin et al., 2004).

**Histopathological assessment:** After euthanasia on day 21st, the hind paws amputated above the knee joint and were fixed in 7.4% formalin solution. The paws were then decalcified using 10% Nitric acid, embedded in paraffin and sectioned in a mid-sagittal plane. The sections of articulation of the tarsal joints were stained with hematoxylin and eosin and were examined microscopically for mononuclear infiltration, pannus formation and bone destruction (Cho et al., 2003; Michele et al., 2005).

**Carageenan induced hind paw edema in rats:** Rats of all the groups were injected 0.1 mL of carageenan (1%) in normal saline into sub planter area of right hind paw. The drugs were given orally 1 h prior to carageenan injection. Paw volume was measured by mercury plethysmograph at 0, 3, 6, 12 and 24 h after the carageenan injection (Ohmayokun et al., 1999).

**Eddy’s hot plate method:** The time for licking paws or jumping in hot plate was recorded as response, prior and 30, 60, 120 and 150 min after administration of Arthosansar/reference standard drug (Somchit et al., 2004).

**Acetic acid induced writhing test in mice:** Writhing was induced 30 min after the last dose by intraperitoneal injection of 10 mL kg⁻¹ of 0.6% acetic acid in distilled water. The number of writhes was counted for 30 min immediately after the acetic acid injection (Nivsarkar et al., 2002). The percentage inhibition of abdominal constrictions between control animals and poly herbal formulation treated animals using the ratio was calculated using formula:

\[
\text{Inhibition (\%)} = \frac{\text{Control mean}-\text{Treated mean}}{\text{Control mean}} \times 100
\]

**Statistical analysis:** All values were reported as Mean±SEM. Results were analyzed using One way ANOVA, followed by Dumnet’s/Tukey’s test, p<0.05 was considered to be statistically significant.

**RESULTS AND DISCUSSION**

As shown in Table 1, CFA treatment caused increase in the paw volume and decrease in the body weight. CFA elevates the levels of ALT, AST and TP. After treatment with AS there is a significant decrease in paw volume, increase body weight and reduction in elevated levels of ALT, AST and TP (Table 2). Histopathology of knee joint of CFA treated rat, reveals enhanced neutrophil infiltration, pannus formation and bone erosion, whereas in AS treated rats, there is significant reduction in neutrophils infiltration, pannus formation and bone.
Table 1: Effect of arthosansar on paw volume and body weight in CFA induced arthritis in rats

<table>
<thead>
<tr>
<th>Treatments</th>
<th>0 day</th>
<th>4th</th>
<th>7th</th>
<th>14th</th>
<th>21st</th>
<th>0 day</th>
<th>4th</th>
<th>7th</th>
<th>14th</th>
<th>21st</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.124±0.002</td>
<td>0.106±0.002</td>
<td>0.106±0.002</td>
<td>0.106±0.002</td>
<td>0.106±0.002</td>
<td>173.34±2.47</td>
<td>176.52±2.32</td>
<td>185.04±2.29</td>
<td>169.64±1.90</td>
<td>194.62±0.20</td>
</tr>
<tr>
<td>Arthritic control</td>
<td>0.124±0.003</td>
<td>0.124±0.003*</td>
<td>0.124±0.002*</td>
<td>0.124±0.002*</td>
<td>0.124±0.002*</td>
<td>150.06±4.47</td>
<td>154.34±4.63</td>
<td>159.54±6.10</td>
<td>161.94±2.10</td>
<td>164.54±2.10</td>
</tr>
<tr>
<td>Arthosansar 500 mg kg⁻¹ p.o.</td>
<td>0.128±0.001</td>
<td>0.128±0.000**</td>
<td>0.128±0.001**</td>
<td>0.128±0.001**</td>
<td>0.128±0.001**</td>
<td>141.36±2.90</td>
<td>140.45±2.25</td>
<td>133.06±2.10</td>
<td>135.34±1.85</td>
<td>142.54±1.60</td>
</tr>
<tr>
<td>Arthosansar 270 mg kg⁻¹ p.o.</td>
<td>0.116±0.004</td>
<td>0.117±0.003</td>
<td>0.117±0.004</td>
<td>0.117±0.003***</td>
<td>0.117±0.004**</td>
<td>173.82±2.88</td>
<td>179.52±2.97</td>
<td>194.93±1.02</td>
<td>185.93±2.93</td>
<td>196.93±3.10</td>
</tr>
<tr>
<td>Standard indomethacin 5 mg kg⁻¹ p.o.</td>
<td>0.124±0.004</td>
<td>0.128±0.002</td>
<td>0.124±0.004</td>
<td>0.128±0.003***</td>
<td>0.124±0.003**</td>
<td>173.04±2.88</td>
<td>181.64±2.75</td>
<td>180.64±2.67</td>
<td>191.64±2.63</td>
<td>195.54±3.10</td>
</tr>
</tbody>
</table>

Values are expressed as (Mean±SEM) N=6. One way ANOVA followed by Tukey's multiple comparison test, *p<0.05 vs control, **p<0.01 vs arthritic control, ***p<0.001 vs arthritic control

Table 2: Effect of polyherbal formulation on lysosomal enzyme and total protein

<table>
<thead>
<tr>
<th>Treatments</th>
<th>ALT (IU)</th>
<th>AST (IU)</th>
<th>TP (g dL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>34.9±0.772</td>
<td>104.8±3.03</td>
<td>12.8±0.685</td>
</tr>
<tr>
<td>Arthritic control</td>
<td>49.8±0.620</td>
<td>145.3±1.145</td>
<td>13.7±0.189</td>
</tr>
<tr>
<td>Standard</td>
<td>21.0±0.738***</td>
<td>65.4±1.264***</td>
<td>9.7±0.546***</td>
</tr>
<tr>
<td>AS 270 mg kg⁻¹</td>
<td>29.7±0.419***</td>
<td>93.1±0.418***</td>
<td>11.9±0.143***</td>
</tr>
<tr>
<td>AS 540 mg kg⁻¹</td>
<td>26.2±0.525***</td>
<td>76.1±1.860***</td>
<td>10.2±0.660***</td>
</tr>
</tbody>
</table>

Values are expressed as (Mean±SEM) N=6. One way ANOVA followed by Dunnet's test, p<0.001 vs control, **p<0.01, ***p<0.001 vs arthritic control

RA is a chronic, cytokine-mediated destructive inflammatory polyarticular joint disease, characterized by massive synovial proliferation, systemic and local inflammation resulting in cartilage and bone destruction. Adjuvant Arthritis (AA) in rat mimics many of the clinical and pathological features of human RA, such as paw swelling, joint erosions and arkylosis and it is the most commonly used animal models for RA (Hong-Meixu et al., 2007).

In the present study, we used AA rats to demonstrate the inhibiting effects of a polyherbal formulation, AS on RA. The in vivo experiments confirmed that Arthosansar (270 and 540 mg kg⁻¹, orally) significantly reduced paw volume and increased the body weight in AA rats. The inhibition of the increase in hind paw volume may be associated with inhibition of neutrophil infiltration, Pannus formation and bone erosion (Aldul-Shakoor et al., 2007). It is supported by histological studies of knee joints. Figure 4 is TS of knee joint of control rat. Severe neutrophil infiltration, Pannus formation and bone erosion is seen in knee joint of Arthritic control rat as shown in Fig. 5. On treatment with AS 270 mg kg⁻¹ there is slight
Fig. 4: TS of Knee joint of control rat

Fig. 5: TS of Knee joint of Arthritic rat showing Mononuclear infiltration, bone erosion

Fig. 6: TS of Knee joint of Indomethacin treated rat
duction in the neutrophil infiltration, Pannus formation and bone erosion but AS 540 mg kg⁻¹ showed significant reduction neutrophil infiltration, Pannus formation and bone erosion, which is comparable with reference standard drug as shown in Fig. 6-8, respectively (Michele et al., 2005). As in AA model, AS decreased the elevated level of lysosomal enzymes which may be due to inhibition of either release or by stabilizing lysosomal enzymes and Cytokines which play key role in the development of inflammation (Vijayalakshmi et al., 1997).

Fig. 7: TS of Knee joint of AS 270 mg kg⁻¹ treated rat

Fig. 8: TS of Knee joint of AS 540 mg kg⁻¹ treated rat showing reduced mononuclear infiltration and bone erosion

The development of edema in the paw of the rat after injection of carrageenan is a biphasic event. The initial phase of the edema has been attributed to the release of histamine and serotonin, the edema maintained during the plateau phase to kinin like substances and the second accelerating phase of swelling to the release of prostaglandin like substances. Inhibition of edema observed in various inflammatory models induced experimentally in the present study may, therefore be attributed to the ability of the Arthosanar to inhibit various chemical mediators of inflammation like histamine and 5-HT during the initial phase (Harsh, 2000).

In the present study, Arthosanar significantly increased the reaction time in hot-plate test suggesting its central analgesic activity, the probable mechanism could be by inhibition of prostaglandin synthesis. Prostaglandins play significant role in different phases of inflammatory reactions and elicited pain by direct stimulation of sensory nerve endings and also sensitize sensory nerve endings to other pain provoking stimuli. Some of constituents like Commiphora mukul, Boswellia serrata, Zingiber officinale of AS possess anti-inflammatory activity (Bagul et al., 2005; Ammon, 2006) but the mechanism of action of these herbal drugs is not yet
proven. Further studies are required on each ingredients of AS to explore the mechanism of anti-inflammatory activity. The peripheral analgesic activity of AS against acute inflammatory pain was good as compared to potent inhibitory activity of indomethacin. Aspirin and Indomethacin often give relief from inflammatory pain by suppressing the formation of pain substances in the peripheral tissues, where prostaglandins and Bradykinin were suggested to play an important role in the pain process (Hajare et al., 2000). Therefore, it is likely that Athosansar might suppress the formation of these substances and they exert its analgesic activity in acetic acid-induced writhing test. Further studies are required on each ingredients of AS to explore the mechanism of analgesic activity.

**CONCLUSION**

Athosansar, a poly herbal formulation possesses anti-arthritis, anti-inflammatory and analgesic property.

**REFERENCES**


