Elucidation of mechanism of Anti-arthritic action of
Arthosansar- a polyherbal formulation

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To elucidate the mechanism of anti-arthritic action of the Arthosansar, a polyherbal formulation has proven anti-arthritic activity. The mechanism is elucidated by emphasizing on immunomodulation, anti-inflammatory and analgesic action. The study is carried out using CFA induced arthritis in rats, carrageenan induced peritonitis in mice, inflammation induced by various phlogistic agents in rats, acetic acid induced writhing in mice and tail-immersion test in mice. Arthosansar significantly (P<0.001) inhibited proliferation of lymphocytes in CFA induced arthritic rats. Arthosansar significantly (P<0.001) inhibited neutrophil infiltration in carrageenan induced peritonitis in mice, inhibited the release of inflammatory mediators (PGs, histamine, 5-HT and bradykinin) and pain mediated through these mediators in acetic acid induced writhing test. Arthosansar also showed significant (P<0.001) increase in latency period in tail immersion test in mice. Arthosansar exhibits anti-arthritic activity by (a) Inhibiting lymphocyte proliferation (b) Inhibiting production of IF-γ, TNF-α, IL-2 (c) Inhibiting release of inflammatory mediators PGs, histamine, 5-HT and bradykinin (d) Exhibits antinociceptive action through opioid receptors and by promoting release of endogenous peptides.

Keywords: Anti-arthritic activity, Immunomodulation, Anti-inflammatory, Analgesic, Neutrophil infiltration

IPC Int. Cl.: A01D 9/00, A01D 9/01, A01D 9/04, A01D 9/08, A01D 9/09, A01D 9/10, A01D 9/11, A01D 4/29, A01D 20/54

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disorder of joints characterized by proliferation of the synovial membrane and persistent uncontrolled inflammation resulting in a chronic destructive polyarthritis. It is an autoimmune disease, where the immune system (the body’s defense system against disease) mistakenly directs its destructive capability against part of the body. Rheumatoid arthritis often begins in joints of the extremities, such as those of fingers and wrists. The autoimmune activity seems to affect the synovial membrane and joints become painful and stiff. The disease is progress to total destruction of the synovial membrane and calcification of the joint. Such a joint is then fused and has no mobility

Therapy has been targeted towards the treatment of the signs and symptoms of the disease as well towards changing its natural history. The therapy includes, non-steroidal anti-inflammatory drugs (NSAID), although known to induce significant damage to the gastrointestinal tract, are still used to treat the primary symptoms, and Disease Modifying Anti-Rheumatic Drugs (DMARDS) like cyclophosphamide, intramuscular gold, anti-malarial therapy, sulfasalazine, methotrexate, etc.

Apart from Allopathy, the alternative medicine to treat arthritis includes herbal drugs with less or no side effects. Arthosansar is proved to be more effective as anti arthritic with less or no side effects. Arthosansar is a polyherbal formulation developed by Pradhan Herbal Company, Bangalore, containing 7 plant constituents, viz. Guggulu [Commiphora wightii Arn], Sallaki [Boswellia serrata Roxb], Rasna [Pluchea lanceolata], Eranda [Ricinus communis Linn.], Shunti [Zingiber officinale], Shilajit (a gummy substance found in the rocks of certain mountainous regions of the world) and Aswagandha [Withani somnifera Linn].

Earlier studies have confirmed that the polyherbal formulation, Arthosansar, has activity against inflammation and algesia associated with arthritis. As a result of these studies the drug is used as anti-arthritic. The present study was undertaken to elucidate the mechanism of anti-arthritic action of the polyherbal formulation, Arthosansar.
Methodology

Experimental animals
Male Wistar rats weighing 175 ± 25 gm and Swiss albino mice weighing 25 ± 5 gm of either sex were used for the study in different models. The animals were procured from authorized institution NIMHANS, Bangalore. Animals were housed in propylene cages and maintained under standard conditions of 27±2°C and relative humidity 65±10 % for two weeks before and during the experiment in institutional animal house, (registration No. 152/1999/CPCSEA). All animals were fed the standard rodent diet (Mysore Feeds Limited, Bangalore) and provided water ad libitum. Institutional Animal Ethical Committee permission was taken before starting the study.

Drugs and chemicals
Arthosansar was a gift sample from Pradhan Herbal Company, Bangalore. Complete Freund’s adjuvant emulsion was obtained from Difco lab, USA, ALT, AST and total protein kits were obtained Span Diagnostics, Bangalore. Cyclophosphamide was obtained from Himedia, Mumbai. Carrageenan was obtained from Sigma labs, Bangalore. Serotonin and Histamine were obtained from Sigma life Sciences, Bangalore. Arachidonic acid and Bradykinin were obtained from Sigma Aldrich, USA All other chemicals and reagents used were of analytical grade obtained from institutional chemical store.

Experimental design

CFA induced arthritis in rats
Male Wistar rats weighing 150-200 gm were divided into 5 groups of 6 animals each. Group I served as normal control, Group II served as arthritic control, Group III animals were treated with Indomethacin (5 mg/kg, p.o), Group IV animals were treated with cyclophosphamide (7 mg/kg, p.o), Group V animals were treated with Arthosansar (540 mg/kg, p.o).

On day 1, arthritis is induced by injecting 0.1ml CFA in the subplanatar region of the left hind paw after one hour of test compound and standard drug administration to the animals of respective groups. Arthosansar and the standard drugs are administered from day 1 and continued up to 12th post CFA challenge day. Paw volume is measured using mercury plethysmograph on day 1, day 5, and day 21. On day 21, along with the paw volume, the severity of secondary lesions is evaluated visually and graded as follows.

**Ear**: absence of nodules and redness (score=0); presence of nodules and redness (score=1),

Nose: no swelling of connective tissue (score=0); intensive swelling of connective tissue (score=1),

Tail: absence of nodules (score=0); presence of nodules (score=1),

Forepaws: absence of inflammation (score=0); inflammation of atleast 1 joint (score=1), Hindpaws: absence of inflammation (score=0); slight inflammation (score=1); moderate inflammation (score=2); marked inflammation (score=3)

For primary lesions: The percentage inhibition of paw volume of the injected left paw over vehicle control is measured at day 5.

For secondary lesion: The percentage inhibition of paw volume of the non-injected right paw over vehicle control is measured at day 21.

An arthritic index is calculated as the sum of the scores as indicated above for each animal. The average of the treated animals is compared with the control group. The total percentage change is calculated as: primary lesion+ secondary lesion+ percent change of the arthritic index.

At the end of 21st day, rats were anaesthetized and blood was withdrawn by puncture of retro orbital plexus for biochemical estimation of serum alanine aminotransferase (ALT), serum aspartate transaminase (AST) and total protein. After euthanasia on day 21st, the hind paws were amputated above the knee joint, fixed using 7.4% formalin solution for histopathological studies.

Carrageenan induced peritonitis (vascular permeability in rats)
Male Swiss Albino mice weighing between 20-25 gm were divided into 4 groups of 6 mice each. Group I served as normal control, Group II served as peritonitis control, Group III animals were treated with Indomethacin (10 mg/kg, p.o), Group IV animals were treated with Arthosansar (800 mg/kg, p.o).

Standard drug Indomethacin was given 1hr prior while the test drug was given for 6 days prior the induction of peritonitis. One hour after the administration of the drugs, carrageenan (0.25 ml of 0.75%-w/v in saline) was injected intraperitonealy. Four hours later, the animals were sacrificed and
Ca/Mg free phosphate-buffered saline was injected into the peritoneal cavity. Following a gentle massage, peritoneal exudates were removed. The total leukocyte count and the differential cell count was determined using Neubauer chamber. The % of leukocyte inhibition and inhibition of neutrophil migration was calculated using the following formulae.

\[
\% \text{ leukocyte inhibition} = (1 - \frac{T}{C}) \times 100
\]

Where, —T represents the leukocyte count of the treated group and —C represents the leukocyte count of the peritonititis control group.

\[
\text{Inhibition of neutrophil migration} = \frac{100 - \left( \frac{NT}{NC} \right) \times 100}{100}
\]

Where, —NT is neutrophil count of the treated group and —NC is the neutrophil count of the peritonitis control group.

**Phlogistic agents induced paw edema**

Male Albino Wistar rats weighing 175-200 gm were divided into 9 groups of 6 rats each. Group I served as normal control, Group II, IV, VI, VIII served as arachidonic acid (0.1 ml of 0.5% solution) induced inflammatory control, histamine (0.1 ml of 0.1% solution) induced inflammatory control, bradykinin (0.1 ml of 0.002% solution) induced inflammatory control and serotonin (0.1 ml of 0.1% solution) induced inflammatory control respectively. Group III, V, VII, IX animals were treated with Arthosansar (540 mg/kg, p.o) 1hour prior the induction of inflammation by injecting arachidonic acid, histamine, bradykinin and serotonin to respective groups. Paw volume was measured using mercury plethysmograph at 0 hr and at 1, 2, 3 and 4hr after administration of phlogistic agents.

**Acetic acid induced writhing test in mice**

Swiss – albino mice weighing 20-25 gm were divided into 4 groups of 6 animals each. Group I served as normal control, Group II served as acetic acid (10 ml/kg, i.p of 0.6% solution) induced writhing control, Group III animals were treated with diclofenac (10 mg/kg, i.p) and Group IV animals were treated with Arthosansar (800 mg/kg, p.o). Arthosansar and the vehicle were administered orally 30 minute before but diclofenac-sodium was administered intraperitonially 15 minute before injection of acetic acid. After an interval of 15 minute, mice were observed for writhes for next 10 minute ant the number of writhes were recorded. The formula for computing per cent inhibition is:

\[
\% \text{ inhibition} = \frac{\text{Average writhes in the control group} - \text{writhes in the drug group}}{\text{writhes in the control group times 100%}}
\]

**Tail immersion in mice**

Swiss Albino mice of either sex weighing 20-25 gm were divided into 3 groups of 6 animals each. Group I served as vehicle control, Group II animals were treated with tramadol (25 mg/kg, i.p), Group III animals were treated with Arthosansar (800 mg/kg, p.o). Arthosansar was administered 60 minute prior while tramadol was administered 30 minute prior the commencement of the test. Distal 1-2 cm of the tail of mice was immersed in hot water kept constant at 55° C. The reaction time (the time taken by the mice to deflect their tails) was recorded. The cut off period was 15sec. The latent period of tail-flick response was determined at 0, 30, 60 and 90 min after the administration of drugs. Percentage protection was determined as follows:

\[
\% \text{ Protection} = \frac{\text{Latency (test) Latency (control)} \times 100}{\text{Latency (control)}}
\]

**Statistical analysis**

The results were expressed as mean ± SEM. All the data’s were analyzed by One-Way ANOVA using Graph pad PRISM, version 5 and Post hock analysis was done by suitable comparison test, viz: Tukey’s multiple comparison test, Dunnet’s test. P<0.05 is considered as statistically significant.

**Results**

**Effect of arthosansar on adjuvant induced arthritis in rats**

Arthosansar significantly (P<0.001) decreased the paw volume as compared to arthritic control on day 1, 5, and 21 respectively, which are comparable with the results due to Indomethacin pretreatment in rats. Cyclophosphamide showed a significant (P<0.001) reduction in paw volume as compared to arthritic control rats on day 21. Arthosansar also significantly (P<0.001) reduced the non-injected right hind limb paw volume measured on day 21 as compared to arthritic control rats which is comparable with the results due to Indomethacin and Cyclophosphamide pretreatment in rats (Table 1). Arthosansar significantly
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(\(P<0.001\)) reduced the formation of secondary lesions as compared to arthritic control rats. Indomethacin significantly (\(P<0.05\)) inhibited the secondary lesions compared to arthritic control. Cyclophosphamide showed significant (\(P<0.001\)) reduction in secondary lesions (Fig. 9). The total percentage change (primary lesion+ secondary lesion+ percent change of the arthritic index) in arthritic condition by Arthosansar was 116.39, comparable to both the standard drugs; Indomethacin (NSAID)-88.52 and cyclophosphamide (immunosuppressant) - 137.33 (Table 2).

CFA significantly (\(P<0.01\)) increased AST, ALT and total protein, whereas Arthosansar significantly (\(P<0.05\)) reduced AST, ALT and total protein when compared to arthritic control rats. The results are comparable with that due to pretreatment with Indomethacin and cyclophosphamide (Figs. 1 & 2).

**Histopathological studies**

TS of knee (synovial) joint of normal control rat showed intact articular hyaline cartilage, subchondrial bone layer and adjacent synovial layer. The synovial layer shows synovial lining cells within normal range (Figs. 3A & 3B). TS of synovial joint of arthritic control rat with distorted articular hyaline cartilage with intact subchondrial bone layer and partially affected synovial layer. The distorted articular cartilage, i.e. bone erosion is replaced by mononuclear inflammatory cells comprising of predominantly lymphocytes along with pale eosinophilic fibrinous material, which indicates the pannus formation. The synovial

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**Table 1**—Effect of Arthosansar on day 5 (as anti-inflammatory) and on day 21 (immunomodulatory) in CFA induced arthritis in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>day 1</th>
<th>day 5</th>
<th>day 21</th>
<th>non-injected right paw volume on day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.4 ± 0.004</td>
<td>0.4 ± 0.003</td>
<td>0.4 ± 0.004</td>
<td>0.4 ± 0.002</td>
</tr>
<tr>
<td>Arthritic Control</td>
<td>0.53 ± 0.003*</td>
<td>0.74 ± 0.004*</td>
<td>0.92 ± 0.003*</td>
<td>0.72 ± 0.002*</td>
</tr>
<tr>
<td>Arth + Indo (5 mg/kg, p.o)</td>
<td>0.43 ± 0.002**</td>
<td>0.41 ± 0.003**</td>
<td>0.41 ± 0.003**</td>
<td>0.6 ± 0.004*</td>
</tr>
<tr>
<td>Arth + CPH (7 mg/kg, p.o)</td>
<td>0.43 ± 0.003*</td>
<td>0.44 ± 0.003*</td>
<td>0.41 ± 0.003**</td>
<td>0.46 ± 0.003**</td>
</tr>
<tr>
<td>Arth + AS (540 mg/kg, p.o)</td>
<td>0.45 ± 0.002**</td>
<td>0.42 ± 0.003**</td>
<td>0.41 ± 0.002**</td>
<td>0.52 ± 0.003**</td>
</tr>
</tbody>
</table>

n=6, Values are expressed as Mean ± SEM, ANOVA and Tukey’s multiple comparison test. +\(P<0.001\) vs control and **\(P<0.001\) and *\(P<0.01\) vs Arthritic control.

**Table 2**—Total percentage change in CFA induced arthritic rats by Arthosansar

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Primary Lesions</th>
<th>Secondary Lesions</th>
<th>Arthritic Index</th>
<th>Total % Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Arthritic Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Arth + Indo (5 mg/kg, p.o)</td>
<td>44.59</td>
<td>16.66</td>
<td>27.27</td>
<td>88.52</td>
</tr>
<tr>
<td>Arth + CPH (7 mg/kg, p.o)</td>
<td>40.5</td>
<td>36.11</td>
<td>60.72</td>
<td>137.33</td>
</tr>
<tr>
<td>Arth + AS (540 mg/kg, p.o)</td>
<td>43.24</td>
<td>27.7</td>
<td>45.45</td>
<td>116.39</td>
</tr>
</tbody>
</table>

\(P<0.001\) reduced the formation of secondary lesions as compared to arthritic control rats. Indomethacin significantly (\(P<0.05\)) inhibited the secondary lesions compared to arthritic control. Cyclophosphamide showed significant (\(P<0.001\)) reduction in secondary lesions (Fig. 9). The total percentage change (primary lesion+ secondary lesion+ percent change of the arthritic index) in arthritic condition by Arthosansar was 116.39, comparable to both the standard drugs; Indomethacin (NSAID)-88.52 and cyclophosphamide (immunosuppressant) - 137.33 (Table 2).

CFA significantly (\(P<0.01\)) increased AST, ALT and total protein, whereas Arthosansar significantly (\(P<0.05\)) reduced AST, ALT and total protein when compared to arthritic control rats. The results are comparable with that due to pretreatment with Indomethacin and cyclophosphamide (Figs. 1 & 2).

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**Fig. 1**—Effect of Arthosansar on serum aminotransferases n=6; Values are expressed as (Mean ± S.E.M); One way ANOVA followed by Tukey’s multiple comparison test. +\(P<0.001\) vs control and **\(P<0.001\) and *\(P<0.01\) vs Arthritic control.

**Fig. 2**—Effect of Arthosansar on serum Total Protein n=6; Values are expressed as Mean ± S.E.M One way ANOVA followed by Tukey’s multiple comparison test. +\(P<0.001\) vs control **\(P<0.01\) ***\(P<0.001\), Vs arthritic control.
subepithelium shows fibrocollagenous stroma with vascular spaces (Figs. 4A & 4B). TS of synovial joint of Indomethacin pretreated arthritic rat showed intact articular hyaline cartilage, subchondrial bone layer and adjacent synovial layer. The synovial layer shows synovial lining cells within normal range. The synovial subepithelium shows fibrocollagenous stroma with vascular spaces. (Figs. 5A & 5B). TS of knee (synovial) joint of cyclophosphamide pretreated arthritic rat showed intact articular hyaline cartilage with intact subchondrial bone layer and synovial layer. The articular cartilage at one focus shows aggregates of mononuclear inflammatory cells. The synovial subepithelium shows fibrocollagenous stroma with vascular spaces (Figs. 6A & 6B). TS of knee (synovial) joint of Arthosansar pretreated rat showed intact articular hyaline cartilage, subchondrial bone layer and adjacent synovial layer. The synovial layer shows synovial lining cells within normal range. The synovial subepithelium shows fibrocollagenous stroma with vascular spaces (Figs. 7A & 7B).

Effect of arthosansar on carrageenan induced peritonitis in rats
Arthosansar significantly (P<0.001) inhibited the leukocyte infiltration compared to carrageenan induced peritonitis control rats (Table 3). Arthosansar also significantly (P<0.001) inhibited neutrophil count compared to carrageenan induced peritonitis control rats. The leukocyte migration and neutrophil infiltration was inhibited by 53.08% and 66.26% respectively by Arthosansar when compared to 64.47% and 77.77% by Indomethacin.

Figs. 3-9—3A)—TS of synovial joint of normal control rat Arrows indicate intact synovial layer. [H & E, 100X]; 3B)—TS of synovial joint of normal control rat. Arrow indicates normal synovial cell lining. [H & E, 400X]; 4A)—TS of synovial joint of arthritis control rat. Arrow indicates distorted hyaline cartilage. (H & E, 100X); 4B)—TS of knee joint of arthritis control rat. a) Arrow indicates infiltrations of mononuclear inflammatory cells. b) Arrow indicates fibrocollagenous stroma. c) Arrow indicates vascular spaces. (H & E, 400X); 5A)—TS of knee joint of Indomethacin pretreated arthritic rat. Arrow indicates intact articular hyaline cartilage. [H & E, 100X]; 5B)—TS of knee joint of Indomethacin pretreated arthritic rat a) Arrow indicates fibrocollagenous stroma. b) Arrow indicates vascular spaces. [H & E, 400X]; 6A)—TS of knee joint of cyclophosphamide pretreated arthritic rat. Arrow shows intact articular hyaline cartilage [H & E, 100X]; 6B)—TS of knee joint of cyclophosphamide pretreated arthritic rat. a) mononuclear inflammatory cells. b) fibrocollagenous stroma. c) vascular spaces. [H & E, 400X]; 7A)—TS of knee joint of Arthosansar pretreated arthritic rat. Arrow shows intact articular hyaline cartilage. [H & E, 100X]; 7B)—TS of knee joint of Arthosansar pretreated arthritic rat. a) Arrow shows synovial lining cells within normal range. b) Arrow shows vascular spaces. [H & E, 400X]; 8)—Effect of Arthosansar on centrally mediated nociception by Tail immersion test in mice n=6, Values are expressed as Mean ± SEM, ANOVA and Dunett’s comparison test ***P<0.001 v/s control.; 9)—Effect of Arthosansar on secondary lesions in arthritic rats on day 21 n=6, Values are expressed as Mean ± SEM, ANOVA and Tukey’s multiple comparison test. +P<0.001 v/s control, ***P<0.001, *P<0.05 v/s Arthritic control.
Effect of arthosansar on phlogistic agents induced inflammation

Arthosansar significantly decreased the paw edema in rats induced by different mediators from the 1st hour. Arthosansar significantly (P < 0.001) inhibited paw edema in rats challenged with arachidonic acid, histamine, serotonin, bradykinin when compared to respective control rats (Table 4).

Effect of arthosansar on peripherally induced pain

Arthosansar decreased significantly (P < 0.001) the number of acetic acid induced writhes by 78.72% as compared to 84.65% by Diclofenac (Table 5).

Tail immersion test in mice

Arthosansar increased the latency period compared to control rats. Arthosansar significantly (P < 0.001) provided the protection at 6.89%, 66.92%, 79.71% and 89.91% at 0, ½, 1 and 1 ½ hour respectively compared to 10.89%, 77.82%, 89.12% and 98.43% protection provided by tramadol (Fig. 8).

Discussion

The pathogenesis of RA involves an aberrant immune response that leads to synovial inflammation and destruction of joint architecture. Inflammation is divided into 3 phases: acute inflammation, the

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Leukocyte Count (cu. mm)</th>
<th>% LI</th>
<th>Neutrophil Count (cu. mm)</th>
<th>% NI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.6±0.05</td>
<td>-</td>
<td>2.1±0.04</td>
<td>-</td>
</tr>
<tr>
<td>Peritonitis Control</td>
<td>50.16±0.34*</td>
<td>27.31±0.51*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indomethacin (10 mg/kg, p.o)</td>
<td>17.82±0.74**</td>
<td>64.47</td>
<td>6.08±0.27**</td>
<td>77.77</td>
</tr>
<tr>
<td>Arthosansar (800 mg/kg, p.o)</td>
<td>20.55±0.34**</td>
<td>53.08</td>
<td>9.21±0.32**</td>
<td>66.26</td>
</tr>
</tbody>
</table>

n=6, Values are expressed as Mean ± SEM, ANOVA and Tukey’s multiple comparison test. + P < 0.001 v/s control.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Paw vol. at 0hr (ml)</th>
<th>Paw vol. at 1hr (ml)</th>
<th>Paw vol. at 2hr (ml)</th>
<th>Paw vol. at 3hr (ml)</th>
<th>Paw vol. at 4hr (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.401±0.004</td>
<td>0.401±0.004</td>
<td>0.4±0.003</td>
<td>0.4±0.003</td>
<td>0.401±0.004</td>
</tr>
<tr>
<td>AA</td>
<td>0.41±0.002</td>
<td>0.52±0.003</td>
<td>0.68±0.003</td>
<td>0.79±0.003</td>
<td>0.88±0.003</td>
</tr>
<tr>
<td>AA+AS</td>
<td>0.41±0.002</td>
<td>0.46±0.003*</td>
<td>0.44±0.002*</td>
<td>0.41±0.002*</td>
<td>0.41±0.002*</td>
</tr>
<tr>
<td>Hist</td>
<td>0.4±0.002</td>
<td>0.49±0.002</td>
<td>0.58±0.002</td>
<td>0.64±0.002</td>
<td>0.7±0.002</td>
</tr>
<tr>
<td>Hist+AS</td>
<td>0.4±0.001</td>
<td>0.47±0.002*</td>
<td>0.44±0.002*</td>
<td>0.44±0.003*</td>
<td>0.44±0.002*</td>
</tr>
<tr>
<td>5-HT</td>
<td>0.41±0.002</td>
<td>0.48±0.003</td>
<td>0.52±0.002</td>
<td>0.58±0.002</td>
<td>0.67±0.002</td>
</tr>
<tr>
<td>5-HT+AS</td>
<td>0.4±0.002</td>
<td>0.45±0.001*</td>
<td>0.43±0.002*</td>
<td>0.43±0.002*</td>
<td>0.43±0.002*</td>
</tr>
<tr>
<td>BK</td>
<td>0.41±0.002</td>
<td>0.51±0.002</td>
<td>0.63±0.002</td>
<td>0.71±0.002</td>
<td>0.8±0.002</td>
</tr>
<tr>
<td>BK+AS</td>
<td>0.4±0.002</td>
<td>0.48±0.003*</td>
<td>0.45±0.002*</td>
<td>0.42±0.001*</td>
<td>0.41±0.002*</td>
</tr>
</tbody>
</table>

n=6, Values are expressed as Mean ± SEM, ANOVA and Tukey’s multiple comparison test. *P<0.001 v/s respective inflammatory control [Note: AA-Arachidonic Acid control; AA+AS- Arachidonic Acid induced inflammation +Arthosansar; Hist- Histamine control; Hist+AS- Histamine induced inflammation +arthosansar; 5-HT- serotonin control; 5-HT+AS- serotonin induced Inflammation +Arthosansar; BK-Bradykinin control; BK+AS- Bradykinin induced Inflammation +Arthosansar]
immune response and chronic inflammation. Acute inflammation is the initial response to injury, mediated by the release of autacoids and usually precedes the development of the immune response. The immune response occurs when immunologically competent cells are activated. The outcome of the immune response leads to chronic inflammation that results in pain and destruction of bone and cartilage.

In CFA induced arthritis in rats, CFA act by prolonging the life time of injected autoantigen, by stimulating its effective delivery to the immune system and by providing a complex set of signals to the immune system, resulting in altered leukocyte proliferation and differentiation (CD4+ lymphocytes) that causes enhanced phagocytosis and secretion of cytokines by mononuclear phagocytes. Mycobacterial component target Mononuclear Phagocytes (MPC’s) and Dendritic Cells (DC’s) to induce the production of monokines, particularly IL-12 and TNF-α. TNF-α is an inducer of other cytokines (IL-6) and chemokines. IL-12 induces IFN-γ and also acts as driving force for directing T-cell differentiation.

In our study, Injection of complete Freund’s adjuvant into the rat paw induced inflammation as primary lesion with a maximum after 3-5 days. Secondary lesions occurred after a delay of approximately 11-12 days, which are characterized by inflammation of non-injected sites (hind leg, forepaws, ear, nose and tail). The inhibition of the increase in hind paw volume on day 5 is associated with inhibition of neutrophil infiltration, the event in the initial phase of inflammation that is inhibited by NSAID’s. Arthosansar significantly reduced swelling of non-injected paw and also inhibited the inflammation of other non-injected sites, the results of which were comparable with cyclophosphamide. The secondary lesions are due to the activation of the immune system by Freund’s adjuvant, where the proliferative T-cells are the precursor for proinflammatory mediators. Anti-inflammatory compounds do not inhibit secondary lesions, which are prevented OR diminished by immunosuppressive agents.

The study revealed that Arthosansar inhibited the activation and proliferation of T-cells there by inhibiting the secondary lesions comparable to cyclophosphamide. Thus, Arthosansar holds inflammatory mediators and immune system as the substrate for its anti-arthritic activity.

Biochemical estimation of serum aminotransferases indicated that CFA increased levels of Aminotransferases. The increase in aminotransferases is due to their release from the cells of the damaged organ, since liver impairment is a feature of adjuvant arthritis. Arthosansar significantly reduced the serum aminotransferases levels. This effect is due to its anti-inflammatory and anti-oxidant property that prevents organ damage. The adjuvant induced arthritis causes changes in plasma protein concentrations that are manifested as an increase in the globulin fraction and decrease in the albumin fraction. It is postulated that during inflammation, the mediators released, histamine, bradykinin and prostaglandins increase the permeability of vascular tissues to albumin leading to reduction in its serum levels. Thus treatment with arthosansar significantly reduced the increased plasma protein levels, i.e. increased the albumin level and decreased the globulin level in arthritic rats which indicates that Arthosansar have a suppressive action on the mediators of inflammation.

Histopathological studies support and justify the anti-arthritic effect of arthosansar. The TS of synovial joint of arthritic control rat revealed, edema formation, degeneration with erosion of the cartilage, and extensive infiltration of inflammatory exudates in the articular surface leading to pannus formation. The TS of synovial joint of cyclophosphamide pretreated rat showed normal articular hyaline cartilage with foci of inflammatory cell aggregates at articular cartilage, since cyclophosphamide has no effect on the primary phase of inflammation involving infiltration of inflammatory cells. TS of synovial joint of Arthosansar pretreated rat showed intact articular cartilage with no aggregates of inflammatory cells, thus preventing the overall inflammatory signs and pannus formation, similar to the TS of synovial joint of indomethacin pretreated rat showed normal articular hyaline cartilage with no cellular infiltrates.

Carrageenan-induced peritonitis is a well characterized experimental model of acute inflammation that permits the quantification and correlation of cellular migration with changes in other inflammatory parameters. The mechanism of carrageenan on peritonitis involves synergistic action involving prostanoids, leukotrienes B4 and other chemotactic agents such as C5a and IL-8, which promote an increase of the vasodilation, plasmatic exudation and recruitment of leukocytes, mainly neutrophils. Reactive oxygen species and proteases...
produced by activated polymorphonuclear neutrophils cause or exacerbate the inflammatory reactions. To investigate the action of Arthosansar on influx of neutrophils the present experimental model was taken up. It is found that, Arthosansar significantly inhibited leukocyte migration and neutrophil infiltration by 53.08% and 66.26% respectively when compared to 64.47% and 77.77% by Indomethacin. This effect of Arthosansar may be due to the inhibition of endogenously generated prostaglandins, leukotrienes and other chemotactic agents that promote cellular migration which also contribute to the inhibition of pannus formation as indicated in histopathological studies.

When tissues are injured, several endogenous substances are produced, in particular, histamine, bradykinin and prostanooids (prostaglandins, prostacyclin, thromboxane and leukotrienes). Histamine, 5-HT, Bradykinin and prostaglandin are established mediators of acute phase of inflammation causing increase in vascular permeability and vasodilation. In the present study, Arthosansar significantly inhibited the paw edema induced by arachidonic acid, histamine, serotonin and bradykinin. These observations reveal that, Arthosansar exhibits anti-inflammatory effect by blocking the inflammation mediated through various inflammatory mediators.

The abdominal constriction response induced by acetic acid is a sensitive procedure to evaluate peripherally acting analgesics. Acetic acid causes pain by liberating endogenous substances such as serotonin, histamine, prostaglandins (PGs), bradykinins and substance P, which stimulate nerve endings. Local peritoneal receptors are postulated to be involved in the abdominal constriction response. The significant reduction (78.72%) in acetic acid-induced writhes by Arthosansar suggests that the analgesic effect is peripherally mediated via the inhibition of synthesis and release of PGs and other endogenous substances.

Tail-flick response is predominantly considered to be selective for centrally acting analgesics while peripherally acting ones are known to be inactive on this kind of painful stimulus. The main groups of drugs which exert their analgesic properties centrally are the opioids, which act within the central nervous system by modifying neural activity associated with pain. Endogenous opioid peptides serve as chemical messengers, neurotransmitters or neurohormones, in a complex inhibitory signaling system. The role of the endogenous opioid peptides in the mechanism of transmission of pain involves the interaction between substance P and methioninenkephalin. Substance P is a neurotransmitter for small fibre afferents in the substantia gelatinosa. Synapsing with the small fibre terminal is an interneurone, whose neurotransmitter is methioninenkephalin. When methioninenkephalin is released it will activate opioid receptors on the c-fibre terminal. Activation of the opioid receptors causes depolarization of the cell membrane which, in turn, prevents the release of substance P. The nerve impulse conveying painful information thus does not progress beyond the synapse. Analgesic effect against thermal noxious stimuli may be elicited through opioid receptors or through modulation of several such neurotransmitters.

It was demonstrated in our study that oral administration of Arthosansar prolonged reaction time, which is an indication of centrally mediated analgesic potential. The result suggests that Arthosansar exhibits antinociceptive activity by central effect mediated via opioid receptors and by promoting the release of endogenous opiopeptides.

Conclusion / Recommendations

It is proposed that, Arthosansar exhibit anti-arhritic action by following mechanism:

1. Inhibits lymphocyte proliferation primarily T-helper cells (Th1).
2. Inhibits production of IF-γ, TNF-α, IL-2 by Th1 cells.
3. Inhibits release of inflammatory mediators (PGs, Histamine, 5-HT and bradykinin) thus prevents mononuclear infiltration and pain mediated by these mediators.
4. Exhibits antinociceptive action through opioid receptors and by promoting release of endogenous peptides.

The above discussed mechanism of Arthosansar (Fig. 10) for its anti-arhritic activity may be attributed to its different plant components.

*Boswellia serrata*, Roxb: Boswellic acid reduce the synthesis of leukotrienes in intact neutrophils by inhibiting 5-lipoxygenase. Also cause immunomodulation by simultaneously inhibiting TH1 and promoting TH2 cytokine production. It reduces the glycosaminoglycan degradation, essential to prevent articular damage.

*Zingiber officinale* Linn.: Has inhibitory effects on COX-2 enzymes and it attenuates COX-1/ thromboxane synthase enzymatic activity.
Withania somnifera Dunal: also known as ashwagandha, inhibits delayed-type hypersensitivity reactions and suppresses enhanced phagocytic activity of macrophages. It also reduces the glycosaminoglycan degradation in articular cartilage.

Commiphora wightii Arn.: The main ingredients of Commiphora wightii are flavonoids which have potent anti-oxidant actions and are reported to inhibit nitric oxide formation.

Ricinus communis Linn. possess analgesic effect and also are effective in both acute and chronic inflammation.

Shilajit Research supports the use of shilajit and in rheumatism. Commiphora wightii Arn and shilajit, are reported to be effective in experimental arthritis induced by mycobacterial adjuvant.

The ayurvedic product is based on the ancient Indian text for alleviation of pain associated with arthritis. The study was taken up with the aim of elucidating the mechanism of anti-arthritic action of the polyherbal formulation. The data generated in this study/results will provide a scientific evidence/basis to prove the efficacy of the ayurvedic formulation.

To common people, it is validation of claim of the formulation as a drug in treatment of arthritis. For researchers, it proposes as well as proves the underlying mechanism of action.

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