Anti-arthritic Property of Sahacharadi Kashayam Against Freund’s Complete Adjuvant Induced Arthritis in Wistar Rats

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ABSTRACT

Introduction: The available modern molecular drugs for the therapy of Rheumatoid arthritis are beset with several side effects and alternative drugs are urgently needed. The present investigation was performed to evaluate the anti-arthritic activity of Sahacharadi Kashayam (SK), an Ayurvedic formulation, against Freund’s complete adjuvant (CFA) - induced arthritis in rats. Methods: In this experimental trial, SK was administered at doses of 0.5, 1.0 and 1.5 ml/kg body weight orally to adjuvant (CFA) induced arthritic rats. The anti-arthritic activity was evaluated by using paw volume, haematological parameters and arthritic biomarkers. The efficacy of the Kashayam was compared with the standard Leflunomide (10 mg/kg) drug. Results: Significant reduction in paw volume and thickness by SK (0.5 ml dose) has been found and there was considerably improvement in haematological parameters and arthritic markers in CFA rats till 14 days. After 14th day SK treatment with doses (1.0 and 1.5ml), however, reoccurrence of inflammation and pathological changes were observed in rats. Conclusion: The study clearly indicated the anti-arthritic role of SK. Future studies, however, are warranted to provide a new approach in relation to the therapeutic dose and treatment period of SK which may eventually lead to the development of a new category of the anti-arthritic agent. Key Words: Anti-arthritic activity, Arthritis; biomarkers, Sahacharadi Kashayam.

INTRODUCTION

Rheumatoid Factor (RF) which is an IgG factor is found in the serum of about 80% of patients with Rheumatoid Arthritis and is present in high titre frequently in patients with relatively severe disease. However, patients with other chronic inflammatory diseases may also have RF in the serum. Rheumatoid arthritis usually causes inflammation at symmetrical joints. In majority of cases the proximal inter-phalangeal joints, metacarpo-phalangeal joints of the hands and the wrists are commonly involved. Other major contenders likely to induce arthritis are, infecting agents such as herpes virus, rubella virus, Epstein-Barr virus and mycoplasma.

Rheumatoid factors can form immune complexes which are taken up by the PMNs and are also thought to activate complements, both mechanisms generating inflammation. Rheumatoid arthritis is a disease with augmented mortality and significant morbidity for which effective therapeutic interventions are clearly needed. The most vital drugs currently used by Rheumatologist are anti-inflammatory, NSAID and corticosteroids. The above drugs cooperate with -ferryl haemoglobin, inflammatory, NSAID and corticosteroids. The drugs currently used by Rheumatologist are anti-inflammatory, NSAID and corticosteroids. The above drugs cooperate with -ferryl haemoglobin, formed by the reaction of H2O2 with haemoglobin, to produce drug-derived radicals instigating oxidative damage in these systems. Today in many countries phytochemicals have replaced synthetic pharmaceuticals and it is important to emphasize that almost 30% of modern pharmaceutical preparations are obtained directly or indirectly from plants.

Sahacharadi Kashayam (SK) addresses vata disorders like joint pain, osteoarthritis, rheumatoid arthritis etc. Anti-inflammatory and anti-arthritic action of this medicine helps to alleviate the various symptoms including improving the blood circulation in the affected areas, body pain and debility. This Kashayam is taken at a dose of 5 – 15 ml diluted with 15-45 ml of water twice daily before food or as directed by physician. There are no reports of any side effects of this medicine. This medicine gets its reference in Ayurvedic standard treatise Sahasrayogam. This medicine is manufactured by Arya Vaidya Sala, Kottakkal, and Sitaram Ayurveda Pharmacy etc.

The constituent plants used for preparation of Sahacharadi Kashayam are Barleria strigosa, Sida cordifolia, Tinospora cordifolia, Cedrus deodara and Zingiber officinale. This formulation is effective in the management of vata rogas like rheumatic arthritis, numbness, back ache etc. Various properties of these medicinal plants in the formulations defined in texts of Ayurveda, like Sangrahi, Rasayana, Arshnashaka, etc., are gaining scientific validity via modern research adopting “reverse pharmacological” approach. To our knowledge this is the first report on the experimental verification of the role of this Kashayam for its anti-arthritic activity against Freund’s adjuvant induced arthritis.

METHODOLOGY

Animals

Female Wistar rats of 6-8 weeks old weighed 160-180 g body were obtained from KMCH College of pharmacy, Coimbatore. All rats were kept at room temperature and allowed to accommodate in standard conditions at 12-hr light and 12-hr dark cycle in the animal house. Animals were given commercial pellet diet and water ad libitum freely all through the study. The experimental procedure was approved by IAEC (Institution of Animal Ethical Committee) of KMCH governed by CPCSEA, Government of India, (Proposal number: 685/po/02/a/ CPCSEA/ DATED 2015/2016).

Drug

Leflunomide was used as standard drug. The drug dissolved in 1% w/v CMC and administered orally using a gavage needle at 10 mg/kg dosage daily for 42 days.

Sahacharadi kashayam

The Kashayam is prepared out of 6.173g of Sida cordifolia, 3.089g of Tinospora cordifolia, 1.543 g of Cedrus deodora and 1.543g Zingiber officinalis. The Kashayam was obtained from Kottakal Ayurveda Sala, Chennai, India.

Induction of arthritis

Arthritis was brought about by a single intradermal injection (0.1 ml) of Freund’s Complete Adjuvant (FCA) containing 1.0 mg dry heat-killed Mycobacterium tuberculosis per millilitre sterile paraffin oil into a pad of the left hind paw of female rats. A 26G needle was used for injection. The swelling paw were periodically examined (up to 42 days) in each paw from the ankle using Digital Vanier Calipers. 7

Experimental design

Rats were divided into 6 groups of six animals in each group. A group receiving only vehicle, was taken as the normal control group. Arthritis was induced for another 5 sets of rats by Freund’s Complete Adjuvant (FCA) containing 1.0 mg dry heat-killed Mycobacterium tuberculosis per millilitre sterile paraffin oil into a pad of the left hind paw of female rats. A 26G needle was used for injection. The swelling paw were periodically examined (up to 42 days) in each paw from the ankle using Digital Vanier Calipers. 7

Evaluation of arthritis

Paw thickness was studied by compressing the joint by revolving the screw of micrometer screw gauge till the pain elicited as shown by leg withdrawal or squeaking. The distance covered by the screw gauge was recorded. 6

RESULTS

Table 1. Effect of Sahacharadi Kashayam on paw volume.

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Only CFA</th>
<th>CFA + Low Dose 0.5ML</th>
<th>CFA + Middle Dose 1ML</th>
<th>CFA + High Dose 1.5ML</th>
</tr>
</thead>
<tbody>
<tr>
<td>0th day</td>
<td>4.11667 ± 0.242785</td>
<td>3.86667 ± 0.168655</td>
<td>4 ± 0.293258</td>
<td>3.8 ± 0.157056</td>
<td>3.71667 ± 0.101379</td>
</tr>
<tr>
<td>7th day</td>
<td>4.11667 ± 0.242785</td>
<td>8.86833 ± 0.303704***</td>
<td>8.81667 ± 0.215432***</td>
<td>8.325 ± 0.297957***</td>
<td>8.19833 ± 0.299902***</td>
</tr>
<tr>
<td>14th day</td>
<td>4.11667 ± 0.242785</td>
<td>7.12833 ± 0.152565***</td>
<td>5.41 ± 0.338103*</td>
<td>4.77833 ± 0.966914*</td>
<td>5.79167 ± 0.103454*</td>
</tr>
<tr>
<td>21st day</td>
<td>4.11667 ± 0.242785</td>
<td>8.16667 ± 0.244495***</td>
<td>6.88333 ± 0.340995***</td>
<td>7.33333 ± 0.308401***</td>
<td>7.13333 ± 0.285968***</td>
</tr>
<tr>
<td>28th day</td>
<td>4.11667 ± 0.242785</td>
<td>10.0433 ± 0.326901***</td>
<td>8.62 ± 0.258147**</td>
<td>7.84667 ± 0.427665**</td>
<td>8.22833 ± 0.279075*</td>
</tr>
<tr>
<td>35th day</td>
<td>4.11667 ± 0.242785</td>
<td>8.86667 ± 0.217603***</td>
<td>7.41333 ± 0.197293***</td>
<td>7.28667 ± 0.341083**</td>
<td>7.44667 ± 0.201902*</td>
</tr>
<tr>
<td>42nd day</td>
<td>4.46667 ± 0.138243</td>
<td>7.76667 ± 0.33632**</td>
<td>7.75 ± 0.319113***</td>
<td>7.68333 ± 0.307047***</td>
<td>8.11667 ± 0.193936***</td>
</tr>
</tbody>
</table>

Values area expressed as mean ± SEM Statistical Significance (p) calculated by one way ANOVA. Values are expressed as mean ± SEM (n=6). p<0.05, p<0.01, p<0.001, statistically significant in comparison with CFA induced rats; *p<0.001 statistically significant in comparison with control group. Freund’s complete adjuvant (CFA), Sahacharadi Kashayam (SK), Leflunomide (LEF).
Figure 1: Paw volume changes in control and drugs treated rats in CFA model. 1A- Normal control rat. 1B – CFA induced rat; 1C – CFA +LEF; 1D – CFA + SK (0.5ml); 1E – CFA + SK (1.0ml); 1E – CFA + SK (1.5ml).

Figure 2: Indicates the Effect of anti-arthritic activity of Sahacharadi Kashayam on paw volume at different intervals.
after 14 days of treatment, a slight decrease in the paw volume can be found whereas after the 35th day of treatment, the rat group showed significant \((p < 0.001)\) upsurge in paw volume. Oral treatment with standard drug increased paw volume till 7th day and reduced volume till 35th day. After 35 days of treatment, there appear to be a significant brought back of pathological changes when compared with the diseased rats. The Kashayam treatment showed augmented paw oedema and joint inflammation at 0.5ml for minimum treatment period which is depicted in Figure 1A – 1E.

Thus, it can be said that, 42 days treatment with Kashayam \((p < 0.001)\) significantly augment CFA induced arthritis symptoms in SK treated group, where, 1.5 ml dosage of Kashayam showed higher paw volume and higher inflammation when compared with diseased group.

**Effect of SK on serum arthritic biomarkers**

Table 2, Figures 3 and 4 Indicate the effects of the experiment on C - Reactive protein and rheumatoid Factor.

**DISCUSSION**

There a need to find out an alternative medicine which not only cures but also will have very less or no side effects. Ayurveda and Siddha systems of medicines are age old treatments for almost any type of disease. Arthritis is a common term used for many diseases that produce either inflammation of connective tissues, specifically in joints or non-inflammatory degeneration of these tissues. Perhaps the most significant inflammatory conditions to affect humanity are the diversities of arthritis and rheumatism diseases. Throughout the world herbal medicines appear to be widely accepted for treatment of these and many other diseases.

Although nonsteroid anti-inflammatory drugs (NSAIDs) and disease modifying drugs (DMARDS) could manage RA symptoms, the reported side effects from its long term use are quite detrimental. Polyherbalism has been given notable importance in Indian traditional system of medicine, due to their therapeutic efficiency. And efficiency of polyherbal formulations was described in ‘Sarangadhar Samhitā’, an Ayurvedic literature by Sarangadhracharya, when combining the multiple herbs in a particular ratio, it will give a better therapeutic effect and reduce the toxicity. Many research articles have proved the efficacy of Ayurvedic drugs for the treatment of Rheumatoid arthritis.\(^{11-16}\) In another study the GC MS analysis of Sahacharadi kashayam was reported showing the presence of the following molecules such as Heptanediamide, N,N'-di-benzoyloxy- Benzoic acid, Phenol, 2-methoxy-4-(1-propenyl), Eugenol, Tetradecanoic acid, 3-Decanone, 1-(4-hydroxy-3-methoxyphenyl)-(Gingerol), Abietic acid, 3-(6-Hydroxy-3,7-dimethyl-octa-2,7-dienyl)-4-methoxyphenol, 5H-Cyclopropa[3,4]benzo-[1,2-]eazulen-5-one, 1,1a,1b,4,4a,7a,8,9,9a-decahydro-7b,9,9a-., Naphtho[2,3-c] furan-1-(3H)-one, 3a,4,9,9a-tetrahydro-6-hydroxy-4-(4-hydroxy-
3-methoxyphenyl)-7-methoxy-,[3*A-(3*aa,4a,9a)]- and Lupeol. These molecules are known to have anti-inflammatory properties. Some other molecules such as 1-Heptatriacotanol ,Gibbane-1,10-dicarboxylic acid, 4a-(hydroxymethyl)-1-methyl-8-methylene-, 1,4a-lactone, 10-methyl ester, (1à,4aà,4bá,10á)-,3-(3-Hydroxy-4-methoxyphenyl)-f-alanine, Hexadecanoic acid, ethyl ester, n-Hexadecanoic acid were reported to have antioxidant properties. Another molecule, Heptanediamide, N, N'-di-benzoyloxy- Benzoic 4-methoxyphenyl)-(1-alanine, Hexadecanoic acid, ethyl ester, n-Hexadecanoic acid were reported to have antioxidant properties. Another molecule, Heptanediamide, N, N'-di-benzoyloxy- Benzoic acid is known as Myo-neuro-stimulant. 21

In yet another report Sahacharadi kashayam was reported to have very good antioxidant properties. 14 Many research articles have proved the efficacy of Ayurvedic drugs for the treatment of Rheumatoid arthritis. 18-21

Chropra et al, 2012, have compared standardized ayurvedic formulations and hydrochloroquine sulphate (HCQS) in the treatment of Rheumatoid arthritis. 21 In one of our recent papers we have reported the efficacy of Kodasuri veravaippa, a Siddha formulation., on Carrageenan induced paw oedema and Cotton pellet induced granuloma in albino rats and the clinical efficacy of Kodasuri veravaippa(a sidhiha formulation) in patients affected by the disease "Keelvayu"( Arthritis). 22,24

The constituent plants of SK are known for their contributory role in arthritis. There are no reports on the medicinal role of Barleria strigosae. But a closer species, Barleria prioritis is reported to be an excellent antioxidant and anti-inflammatory plant. 25-27 Devadaru (Cedrus deodara (ROXB.) LOUD, according to Susrutha controls vata and kapha balance and is considered a wonder drug plant with various medicinal roles. 22 Thus, it seems quite pertinent that the constituent plants do contribute to the antiarthritic properties of Sahacahradi kashayam. So, in the current study SK showed protection against arthritis at minimal dose and less treatment period when the treatment prolongs with higher dosage the reoccurrence of arthritis was noted in rat model. Therefore, the reports couldn't provide strong evidence for the antiarthritic activity of SK in the current research trials.

CONCLUSION

In conclusion, SK observes to exert beneficial effects at minimal dose and less treatment period on various pathological manifestations of Freund's Complete Adjuvant induced arthritis. But further study is needed to prove clinical effects of this Ayurvedic formulation and to reduce the reoccurrence of arthritis inflammatory changes. However, the present study authenticates the medical benefits of this Kashayam in the treatment of arthritic conditions as reported in literature.

REFERENCES

8. Mehta A, Sethiya N, Mehta C, Shah G. Anti-arthritis activity of roots of Sida cordifolia is known for its anti-inflammatory and analgesic activities. 26 Tinospora cordifolia is a wonder drug plant with various medicinal roles. 22 Thus, it seems quite pertinent that the constituent plants do contribute to the antiarthritic properties of Sahacahradi kashayam. So, in the current study SK showed protection against arthritis at minimal dose and less treatment period when the treatment prolongs with higher dosage the reoccurrence of arthritis was noted in rat model. Therefore, the reports couldn't provide strong evidence for the antiarthritic activity of SK in the current research trials.

Table 3: Effect of anti-arthritic activity of (42 days) of Sahacharadi Kashayam on haematological parameters.

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Only CFA (Standard)</th>
<th>CFA + LF</th>
<th>CFA + (Low dose) SK 0.5ML</th>
<th>CFA + SK (Mid Dose) 1ML</th>
<th>CFA + SK (High Dose) 1.5ML</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rbc (x 10^6/mm^3)</td>
<td>5.45 ± 0.117132</td>
<td>4.56333 ± 0.13674**</td>
<td>5.61667 ± 0.14172</td>
<td>5.70333 ± 0.289006</td>
<td>4.53 ± 0.0966092**</td>
<td>3.77 ± 0.184065**</td>
</tr>
<tr>
<td>Wbc (x 10^9/ m^3)</td>
<td>10.1 ± 0.255604</td>
<td>9.33333 ± 0.327278**</td>
<td>11.9 ± 0.256472</td>
<td>13.8333 ± 0.586437**</td>
<td>13.6333 ± 0.585757***</td>
<td>3.3333 ± 0.33127***</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>13.333 ± 0.0210819</td>
<td>10.7 ± 0.350782</td>
<td>13.8667 ± 0.406612**</td>
<td>14.06067 ± 0.423215</td>
<td>10.6 ± 0.289828**</td>
<td>9.5 ± 0.324551***</td>
</tr>
<tr>
<td>Polymorphs (%)</td>
<td>4.3333 ± 0.210819</td>
<td>8.33333 ± 0.133333</td>
<td>12.66667 ± 0.918937***</td>
<td>12.66667 ± 0.95571*</td>
<td>13.6667 ± 0.91555***</td>
<td>3.3333 ± 0.921637</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>90.3333 ± 0.557773</td>
<td>85 ± 0.966092*</td>
<td>77.3333 ± 1.11555***</td>
<td>87.3333 ± 0.557773</td>
<td>87.6667 ± 2.01108</td>
<td>88.6667 ± 1.11555</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>2.66667 ± 0.0210819</td>
<td>3.33333 ± 0.210819</td>
<td>3.66667 ± 0.210819</td>
<td>4 ± 0.210819</td>
<td>2.66667 ± 0.210819</td>
<td>2.66667 ± 0.210819</td>
</tr>
</tbody>
</table>

Freund's complete adjuvant (CFA), Sahacharadi Kashayam (SK), Leflunomide (LEF). Values are expressed as mean ± SEM (n=6). *p<0.05, **p<0.01, ***p<0.001, statistically significant in comparison with CFA induced rats; #p<0.05, ##p<0.01, ###p<0.001 statistically significant in comparison with control group.


**GRAPHICAL ABSTRACT**

**SUMMARY**

Paw volume changes in control and drugs treated rats in CFA model. 1A- Normal control rat. 1B – Freund’s complete adjuvant (CFA) induced rat; 1C – CFA + Leflunomide (LEF); 1D – CFA + Sahacharadi Kashayam (SK) (Low Dose-0.5ml); 1E – CFA + SK (Mid dose-1.0ml); 1E – CFA + SK (High dose-1.5ml).