A study to evaluate anti-oxidant and anti-inflammatory activity of curcumin longa and fenugreek in osteoarthritis – A randomized, triple blind, placebo controlled clinical trial

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Abstract: Background: Osteoarthritis (OA), is a leading causes of disability. Herbal medications are often used as an alternative to allopathic drugs to manage symptoms of osteoarthritis. Objectives: This study was conducted to evaluate the efficacy of curcumin longa & fenugreek extracts in patients of KOA (knee osteoarthritis). Methods: Patients were randomized into four groups. Group 1 received placebo (glucose 500mg), Group 2 curcumin longa (CL 500mg), Group 3 Fenugreek (FE 500mg) and Group 4 combination (CL 500 mg + FE 500 mg). Also, aceclofenac 100 mg twice daily was given to all patients. Groups were compared using VAS & WOMAC scores, as well as, by changes seen in serum level of IL-1β and SOD at 0, 60 and 120 days. Results: VAS and WOMAC scores, IL - 1β level significantly reduced, while SOD level significantly increased in CL, FE, and (CL+FE) groups as compared to placebo at 60 and 120 days. Though the effect in FE group is not significant in 60 days, significant improvement was seen at 120 days. Conclusion: Curcumin longa and Fenugreek may be effective in reducing progression and pain of OA, and can be combined with NSAIDs. They are not known to produce any side effects and have better tolerability.

Keywords: NSAIDS, VAS, WOMAC, SOD, Arthritis, Curcumin Longa, Fenugreek.

Introduction

Osteoarthritis (OA) refers to a clinical syndrome of joint pain with multifactorial etiopathogenesis that is characterized by the gradual loss of articular cartilage, osteophyte formation, subchondral bone remodelling, and inflammation of the joint [1].

Among those over 60 years old, about 10% of males and 18% of females are affected. Due to the increase in the ratio of ageing population, the prevalence of OA is expected to increase in the next few decades. It is now seen to affect relatively younger populations of the age group 50 to 60 years. Elderly subjects with chronic joint pain due to OA have a significantly lower quality of life. Weight gain causes an increase in osteoarthritis, which leads to increase immobility and thus again weight gain and vicious cycle sets up leading to increased joint degradation. Although OA may affect any joint in the body, it most commonly affects the knee joint followed closely by the hip joint [2]. OA is not just cartilage problem rather whole joint is involved in it [3-4].

Cytokines and chemokines play an important role in the process of joint destruction in OA by recruiting more inflammatory cells like macrophages. The risk of OA almost doubles in people suffering from obesity [5]. Earlier OA was often considered a non-inflammatory joint problem, but now many studies have proven that there is a big role of inflammatory mediators in the progression of OA [4].
Allopathic medicines provide relief in osteoarthritis but are associated with a lot of side effects. In contrast, Ayurveda offers “safe and effective treatment alternatives” for OA.

Curcumin longa had shown the most promising results in previous studies [2, 6]. While fenugreek has been used for centuries for management of pain and inflammatory conditions with no side effects and has also shown good results in animal studies [7]. Thus, we decided to put fenugreek and “curcumin longa + fenugreek” combination for clinical trial for the treatment of OA in our study.

The objectives of this study were to evaluate the role of curcumin longa and fenugreek in patients of KOA (Knee Osteoarthritis) using Visual Analogue Scale (VAS) and functional status (pain, stiffness, and physical disability) by the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC). Also, changes in IL-1β as an inflammatory marker and superoxide dismutase (SOD) as an antioxidant in serum of the selected patient at 0, 60 & 120 days.

Material and Methods

The study was conducted at the Department of Pharmacology & Therapeutics in collaboration with the Department of Rheumatology and Centre for Advance Research, King George Medical University, Lucknow, India. The study as approved by ‘The Institutional Ethics Committee, King George’s Medical University (Ref. code: 93rd ECM II B-Thesis/ P34). The study was designed as a single centered, triple-blinded, multi-arm, randomized, placebo-controlled clinical trial. The study was conducted according to CONSORT (Consolidated Standards of Reporting Trials) guidelines for reporting RCTs.

Patients Selection and Eligibility Criteria: Patients were enrolled from OPD of the Department of Rheumatology at King George’s Medical University who presented for knee OA and sign the consent for trial in Hindi /English language. Patients were selected and graded based on KL (Kellgren and Lawrence) and randomly divided into four groups [2].

- **Group I** - Aceclofenac 100mg + Placebo (glucose) (twice a day)
- **Group II** - Aceclofenac 100mg + CL 500mg (twice a day)
- **Group III** - Aceclofenac 100mg + FE 500mg (twice a day)
- **Group IV** - Aceclofenac 100mg + CL 500mg + FE 500mg (twice a day)

All of the curcumin longa extract, fenugreek extract, and placebo was given in the form of capsules, which were made under the supervision of qualified persons using standard techniques in the Department of Pharmacology, KGMU, Lucknow. Capsules were similar-looking and were of capacity 1000mg and size ‘00’(1000mg capacity). Along with this, every patient took aceclofenac 100mg (Zerodol) twice a day as standard treatment during the study. Two weeks of aceclofenac 100mg (Zerodol) BD was taken by the patient before the initiation of add-on drugs in respective groups, in an attempt to bring baseline parameters at the same level. The same brand of drugs was maintained throughout the study to maintain the same baseline characters. Rabeprazole 20mg was provided to a few patients who developed symptomatic gastritis during the treatment.

Dose determination was done based on prior clinical studies and animal studies [2,7-8] 95% curcumin longa extract and 10% fenugreek extract of very good quality were used in the study. IL-1β ELISA kit (Ray Biotech, USA) which is available commercially was used. SOD was evaluated using a method used in previous studies [9-10]. 3ml of blood sample of the patient was collected and checked for levels of IL-1β, SOD at 0, 60 & 120 days using appropriate techniques. During the entire study period, patients were in contact with us through phone to solve any confusion regarding the study and to ensure proper compliance.

Inclusion Criteria:

1. Both males and females between the age group 40 to 80 years, suffering from primary KOA, who accepted to participate in the study.
2. 2 weeks of aceclofenac 100mg (Zerodol) BD was taken by the patient before the
trial, in an attempt to bring baseline parameters at the same level.
3. Patients with only KL grade 2 and KL grade 3 were included in the study.

Exclusion Criteria:
1. Patients aged less than 40 years or more than 80 years
2. Grade 4 patients were excluded because due to severe damage to joint non-pharmacological interventions and other allopathic interventions may be required, to provide immediate relief to the patient. And it was unethical to make patients devoid of immediate relied. This may affect the result of study and drop out may occur more frequently as ayurvedic medicines take time for their action.
3. Grade 1 patients were excluded as mild cases may show exaggerated responses and bias the results.
4. Secondary or inflammatory arthritis.
5. Moderate or severe synovitis.
6. Traumatic tear of the meniscus.
7. Chronic renal or liver disease.
8. Diabetic, Rheumatoid arthritis, Hypertension, Gastrointestinal, cardiovascular, endocrine or nervous system, or any other systemic disease patient.
9. Allergic history or drug intolerance.
10. NSAIDs or analgesics (other than aceclofenac) used within 2 weeks before the start of the study.
11. Glucosamine sulphate, chondroitin sulphate use.
12. Intra-articular hyaluronate, or systemic or intra-articular glucocorticoids used within 3 months before the study.

Procurement of Study Products: Commercially available curcumin longa extract and Fenugreek extract were recruited from Lavanya Agro-industry, Noida. Both curcumin longa, fenugreek extract will be procured from Lavanya Agro Industries, Noida, UP, INDIA

Batch Number and Shelf Life: Curcumin longa was of 95% extract with batch no: CAI/CUR/110705. The manufacturing date of the product was June 2018. The expiry date of the product was May 2021. Fenugreek was of 10% extract with batch no: CAI/FEN/110735. The manufacturing date of the product was July 2018 and the expiry date was June 2021.

Randomization: Before the start of the study, a Treatment Randomization Code was generated and was kept secret. This code was provided to the Principal Investigator after all the patients completed the treatment. It contained information regarding the content and encoding of placebo, CL, FE, and (CL+FE) capsules. Containers were labelled by the Treatment Randomization Code generated using the random number generator in Microsoft Excel. Four groups were randomly distributed in numbers from 1 to 106 using a random number table in excel. Group I corresponded to placebo, Group II to CL, Group III to FE, Group IV to (CL+FE). Placebo, CL, Fe, (CL+FE) were assigned to containers for groups Group I, Group II, Group III & Group IV respectively, and the treatments were encoded by a qualified person (QP) for the entire course of the study. The Treatment Randomization Code was kept by the QP until the study was finalized.

Blinding: Triple blinding for trial subjects was performed by using labelled jars containing capsules with an identical appearance. Study medication was directly delivered to the OPD of Rheumatology, KGMU, Lucknow pre-labelled and coded according to the randomization list. The randomization code was kept secret from the investigators, physicians, and patients. This code was revealed only after the termination of the study. For possible Dropouts, an extra 10% of patients were included in the study at the start of trial.

Measurement of knee pain by VAS (Visual Analogue Scale): VAS is used for measurement of the severity of pain. The patient marks no pain, mild pain, moderate pain, and severe pain, on the pain chart [11].

Assessment of Functional Status of Koa Patients By Womac: All three parameters of WOMAC (pain, stiffness, physical fitness) were evaluated and studied separately to evaluate improvement to individual parameters rather than the whole WOMAC score [12].
Sample Size Estimation: As per the results of the previous RCT, in a four-month trial on KOA patients, we expected to achieve a 37% reduction (effect size) in the WOMAC score [6]. To detect this, 1 SD (standard deviation), 5% margin error ($\alpha=0.05$), and 80% power (1-$\beta=0.90$) is required to get the minimal sample size per group, using G power software. Therefore subjects required for the study to see a 37% reduction in IL-1$\beta$ from curcumin longa drug in Indian OA patients will be a minimum of 24 per group, making a total of minimum 24 x 4 = 96 patients will be required. Considering 10% dropouts, total patients will be 96 + 9.6 = 106. Study plan algorithm is briefly described in Figure 1.

**Biochemical Estimations:** IL-1$\beta$: IL-1$\beta$ was quantitatively estimated in the serum of patients by using a commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kit, according to the manufacture's protocol (Raybiotech, HUMAN IL-1$\beta$ kit) with sensitivity 0.3pg/ml.

SOD: SOD estimation was done using a method used by Gavali et al. in 2013 [10].

**Statistical Analysis:** It was done using SPSS version 21.0 (Chicago, USA) and Graph Pad Prism 8. Mann Whitney U test, Kruskall-Wallis test were used for intergroup comparison between groups while the Wilcoxon Signed Rank Test was used for intragroup comparison as VAS scale and WOMAC scale. ANOVA test was used for intergroup comparison of IL-1$\beta$ and SOD. While Paired t-test was used for intragroup comparison of IL-1$\beta$ and SOD as this data was parametric. P-value <0.05 was considered significant for this data analysis.

**Results**

The age of patients enrolled in the study ranged from 47 years to 63 years, with a mean ± SD of 60.55±6.10. While BMI of patients enrolled in the study ranged from 26 Kg/m2 to
33 Kg/m², with mean ± SD of 29.06±2.51. For 103 patients who completed the study Group I, Group II, Group III, Group IV had 26, 25, 25, 27 patients respectively. Out of 103 patients enrolled in the study, 52 were Grade 3, and the rest 51 were of Grade 2.

### Table 1: Intergroup Comparison of Pain (VAS) at different time intervals

<table>
<thead>
<tr>
<th>Time Int.</th>
<th>Group I (n=26)</th>
<th>Group II (n=25)</th>
<th>Group III (n=25)</th>
<th>Group IV (n=27)</th>
<th>Kruskall-Wallis test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Md</td>
<td>Mn</td>
<td>SD</td>
<td>Md</td>
<td>Mn</td>
</tr>
<tr>
<td>Day 0</td>
<td>7</td>
<td>7.08</td>
<td>0.93</td>
<td>7</td>
<td>7.40</td>
</tr>
<tr>
<td>Day 60</td>
<td>6.0</td>
<td>6.04</td>
<td>1.08</td>
<td>4</td>
<td>4.52</td>
</tr>
<tr>
<td>Day 120</td>
<td>5</td>
<td>5.19</td>
<td>1.02</td>
<td>3</td>
<td>3.12</td>
</tr>
</tbody>
</table>

On Day 0, baseline VAS pain score (Day 0) of patients of the above groups was found to be comparable. Order of pain (VAS score) on follow up at Day 60 was Group I > Group III > Group II ≈ Group IV. Order of pain (VAS score) on follow up at Day 120 was Group I > Group III > Group II > Group IV. In all the group's subsequent decline in baseline pain (Day 0) was observed on Day 60 and Day 120, and was found to be statistically significant (Table 1).

### Table 2: Intergroup Comparison of WOMAC ‘Pain score’ at different time intervals

<table>
<thead>
<tr>
<th>Time Int.</th>
<th>Group I (n=26)</th>
<th>Group II (n=25)</th>
<th>Group III (n=25)</th>
<th>Group IV (n=27)</th>
<th>Kruskall-Wallis test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Md</td>
<td>Mn</td>
<td>SD</td>
<td>Md</td>
<td>Mn</td>
</tr>
<tr>
<td>Day 0</td>
<td>15.5</td>
<td>15.38</td>
<td>1.94</td>
<td>15</td>
<td>15.36</td>
</tr>
<tr>
<td>Day 60</td>
<td>14</td>
<td>13.88</td>
<td>1.97</td>
<td>11</td>
<td>10.56</td>
</tr>
<tr>
<td>Day 120</td>
<td>13</td>
<td>12.96</td>
<td>1.87</td>
<td>8</td>
<td>7.80</td>
</tr>
</tbody>
</table>

Baseline Pain on the WOMAC scale (Day 0) of patients of the above groups was found to be comparable. At follow up on Day 60 days, Pain on WOMAC scale was maximum in Group III followed by Group I while minimum Pain on the WOMAC scale was observed in Group IV followed by Group II. On follow up at 120 days, Pain on WOMAC scale was maximum in Group I followed by Group III while minimum Pain on the WOMAC scale was observed in Group IV followed by Group II (Table 2).

### Table 3: Intergroup Comparison of WOMAC ‘Stiffness score’ at different time intervals

<table>
<thead>
<tr>
<th>Time Int.</th>
<th>Group I (n=26)</th>
<th>Group II (n=25)</th>
<th>Group III (n=25)</th>
<th>Group IV (n=27)</th>
<th>Kruskall-Wallis test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Md</td>
<td>Mn</td>
<td>SD</td>
<td>Md</td>
<td>Mn</td>
</tr>
<tr>
<td>Day 0</td>
<td>6.0</td>
<td>6.00</td>
<td>1.10</td>
<td>7.0</td>
<td>6.36</td>
</tr>
<tr>
<td>Day 60</td>
<td>6.0</td>
<td>5.62</td>
<td>0.98</td>
<td>5.0</td>
<td>5.12</td>
</tr>
<tr>
<td>Day 120</td>
<td>5.0</td>
<td>5.31</td>
<td>1.01</td>
<td>4.0</td>
<td>4.28</td>
</tr>
</tbody>
</table>
The median baseline WOMAC score for stiffness of all groups was found to be comparable. On day 60 order of stiffness (WOMAC score) was: Group IV < Group II = Group III = Group I. On day 120 order of stiffness (WOMAC score) was: Group IV < Group II < Group III = Group I (Table-3).

### Table-4: Intergroup Comparison of WOMAC ‘Physical Fitness Disability score’ at different time intervals

<table>
<thead>
<tr>
<th>Time Int.</th>
<th>Group I (n=26)</th>
<th>Group II (n=25)</th>
<th>Group III (n=25)</th>
<th>Group IV (n=27)</th>
<th>Kruskall-Wallis test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Md Mn SD</td>
<td>Md Mn SD</td>
<td>Md Mn SD</td>
<td>Md Mn SD</td>
<td>H ‘p’</td>
</tr>
<tr>
<td>Day 0</td>
<td>48 50.54 5.67</td>
<td>52 50.04 4.38</td>
<td>56 52.48 4.13</td>
<td>56 51.07 5.94</td>
<td>1.923 0.589</td>
</tr>
<tr>
<td>Day 60</td>
<td>42 44.27 5.13</td>
<td>41 39.48 4.56</td>
<td>45 43.72 3.49</td>
<td>38 35.15 5.78</td>
<td>32.355 &lt;0.001</td>
</tr>
<tr>
<td>Day 120</td>
<td>37 40.31 5.97</td>
<td>31 33.04 5.47</td>
<td>39 37.72 3.81</td>
<td>24 25.33 4.58</td>
<td>58.385 &lt;0.001</td>
</tr>
</tbody>
</table>

Baseline WOMAC score for Physical fitness disability score of the above groups was found to be comparable in all groups. On day 60 order of Physical fitness disability score (WOMAC score) was: Group IV < Group II < Group III ≈ Group I. On day 120 order of Physical fitness (WOMAC score) was: Group IV < Group II < Group III = Group I (Table-4).

### Table-5: Intergroup Comparison of ‘IL-β levels’ and ‘SOD levels’ at different time intervals

<table>
<thead>
<tr>
<th>Time Int.</th>
<th>Group I (n=26)</th>
<th>Group II (n=25)</th>
<th>Group III (n=25)</th>
<th>Group IV (n=27)</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mn SD</td>
<td>Mn SD</td>
<td>Mn SD</td>
<td>Mn SD</td>
<td>F ‘p’</td>
</tr>
<tr>
<td>IL-β</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>134.50 3.04</td>
<td>136.84 3.34</td>
<td>136.40 3.43</td>
<td>134.15 6.81</td>
<td>2.326 &lt;0.079</td>
</tr>
<tr>
<td>Day 60</td>
<td>103.88 7.36</td>
<td>71.76 8.25</td>
<td>85.20 4.64</td>
<td>55.30 6.54</td>
<td>239.83 &lt;0.001</td>
</tr>
<tr>
<td>Day 120</td>
<td>76.04 8.38</td>
<td>43.56 5.92</td>
<td>51.76 4.39</td>
<td>25.93 3.04</td>
<td>343.38 &lt;0.001</td>
</tr>
<tr>
<td>SOD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>2.628 0.150</td>
<td>2.646 0.141</td>
<td>2.623 0.195</td>
<td>2.641 0.203</td>
<td>0.093 &lt;0.963</td>
</tr>
<tr>
<td>Day 60</td>
<td>2.903 0.153</td>
<td>3.276 0.169</td>
<td>3.172 0.144</td>
<td>3.345 0.147</td>
<td>41.882 &lt;0.001</td>
</tr>
<tr>
<td>Day 120</td>
<td>2.986 0.146</td>
<td>3.499 0.135</td>
<td>3.352 0.146</td>
<td>3.654 0.023</td>
<td>142.696 &lt;0.001</td>
</tr>
</tbody>
</table>

Baseline (day 0) mean IL-β levels above groups were found to be comparable day 0. Order of IL-β levels on day 60 & 120 was the same: Group IV < Group II < Group III < Group I (Table-5). Baseline (day 0) mean SOD levels above groups were found to be comparable. Order of SOD levels on day 60 & 120 was the same: Group IV > Group III > Group II > Group I (Table-5).

**Discussion**

In our study, we found that many patients of the age group between 45 to 60 years are suffering from OA. This shift in age of onset of OA towards a relatively younger population was also described by Bhatia et al in their study [13]. Altogether 57 females and 46 males participated in the study. A review of some previous studies as by Srivastava et al. and Bhatia et al, suggest that OA is more prevalent in women than in men [2, 13]. In our study, we did not found a significant difference between the number of females and males as documented in most of studies in the past.

WOMAC score showed a significant reduction in pain in (CL +FE) group, CL group as compared to placebo at both days 60 and 120. Though the reduction in pain
between the FE group & Placebo was not significant at 60 days but pain reduction was significant in the FE group at 120 days as compared to the placebo group. This concludes that a longer duration of treatment is required with the FE group. Our results are in agreement with the study done by Bannuruet al where using 1000mg/day of curcuminois mixture showed a significant reduction in pain by using VAS & WOMAC score in the CL group as compared to the placebo group at end of 6 months [8]. Haroyan et al. also demonstrated that the WOMAC score was significantly reduced by using curcumin longaand boswellia [6].

A study by Suresh et al. on Freund’s adjuvant-induced arthritis in Albino rats provided a significant reduction in levels of interleukin levels and suggested antioxidant and anti-inflammatory activity of fenugreek [14]. Thus, our study found the same changes in VAS and WOMAC scores as noted by previous studies. The reduction in stiffness in the WOMAC score on days 60 and 120 was significantly higher in the (CL+FE) group as compared to the placebo group. However difference in ‘reduction of stiffness’ was not significant between the CL group, FE group & placebo group on 60 days and between-group FE and Placebo at 120 days.

Reduction in physical fitness disability score using WOMAC was significantly higher in(CL+FE) group, CL group & FE group as compared to the placebo group at both 60 and 120 days. Thus, WOMAC reveals that maximum benefit was received in (CL+FE) group & minimum in placebo group. These findings are also supported by Srivastava et al. in their studies using VAS and WOMAC scores [2, 6]. Our study finding also closely relate with the study of Sindhu et al on rats. In this study, they showed a reduction of paw edema in rats using fenugreek [7].

As per intragroup comparison by VAS scale for pain and WOMAC score (for each of the three parameters pain, stiffness and physical fitness disability) a significant decline in baseline parameter of day 0 was seen at 60 and 120 days in all groups i.e. (CL+FE), CL, FE, placebo. These findings are supported by studies of Srivastava et al, Haroyan et al and Gupte et al [2, 6, 15].

As compared to placebo, all groups (CL+FE) group, CL group, FE group showed a significant reduction in levels of IL-1β at both 60 and 120 days. This reduction in levels of IL-1β is as per studies done by Srivastava et al and Haroyan et al [2, 6]. In a study done by Sindhu et al. using 400mg/kg of fenugreek showed a significant decrease in levels of IL-1β, IL-1α, IL-2, IL-6, and TNFα levels on Freund’s Adjuvants-induced arthritis in albino rats [7].

Results of our study show that an increase in the mean serum levels of SOD in patients serum were significantly higher in (CL+FE) group followed by that of CL group, FE group as compared to the placebo group at both Day 60 and Day 120. This shows that significant antioxidant activity is present in curcumin longa and fenugreek extracts as compared to placebo. This increase in SOD is also supported by studies Srivastava et al and by Suresh et al using fenugreek in albino rats, who showed an increase in the level of SOD after treatment [2, 14].

**Conclusion**

In our study, we found that curcumin longa and fenugreek are not only effective in reducing the symptoms of the disease but are also devoid of any side effects. Results using VAS and WOMAC scores show that both curcumin longa and fenugreek reduce the symptoms in OA and provide significant relief in pain and joint function as compared to placebo.

Curcumin longa and Fenugreek also act as potent anti-inflammatory anti-oxidant properties agents as shown by changes in serum level of IL-1β and SOD. Through this study, we can assume that both fenugreek and curcumin longa may be beneficial for OA in their extract form and should preferentially be given in combination with each other or with NSAIDS for faster relief depending on the severity of disease. However further studies are required to establish the results of our study. Thus, on the basis of our results we can conclude that both curcumin longa and fenugreek are effective in slowing the progression, and reducing the pain in OA; and have synergistic effect with NSAIDS. They
don’t produce any side effects and hence have better tolerability.

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References


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