Evaluation of the anti-inflammatory effect of Yograj Guggul: an in vitro study

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Introduction

Yograj Guggul (YG) is a poly-herbal formulation extensively used by Ayurvedic practitioners to treat inflammatory conditions, such as rheumatism, osteoarthritis, cervical and lumbar spondylosis etc [1]. It contains Sonthi (Zingiber officinale), Pippali (Piper longum Linn), Pippalimula (Piper longum Linn), Chavya (Piper retrofractum), Chitraka (Plumbago zeylanica Linn), Hingapharta (Ferula narthexs Bioss), Ajamoda (Trachyspermum ammi L) Sprague), Sarshapa (Brassica campestris Linn.), Swetajiraka (Cuminum cyminum Linn.), Krishna jiraka (Carum carvi (Linn.), Nirgundi (Vitex negundo Linn.), Indrayava (Halarrhena antidysenterica Roxb.exFlem.Wall), Patha (Cissampelos pareira Linn. Hirsute (DC) Forman), Vidanga (Embelia ribes), Gajapipali (Scindapsis officinalis (Roxb.) Schott), Katuka (Picrorhiza kurroa Royle ex Benth), Ativisa (Aconitum heterophyllum Wall.),Bharangi (Clerodendrum serratum Linn.) Moon), Vacha (Acorus calamus Linn.), Murva (Marsdenia tenacissima Roxb. Moon), Haritaki (Terminalia chebula Retz.), Bibhitaki (Terminalia bellica Gaertn. Roxb.), Amalaki (Phyllanthus emblica Linn.) in an equal proportion along with Guggul (Commiphora wightii). Guggul, which is added in a quantity equivalent to the total quantity of all the herbs, constitutes a major part of the formulation. Guggul has been reported to have anti-inflammatory activity [2] and has also been shown to be as effective as ibuprofen in an animal model of acute and chronic inflammation [3]. However there are no reports available on the anti-inflammatory effect of the whole formulation. The current therapeutic approaches to treat inflammatory diseases are centered on inhibition of cyclooxygenase (both COX-1 and 2) pro-inflammatory enzymes. Although this approach halts the inflammatory process, it brings out activation of the LOX pathway producing Leukotrienes. The Leukotrienes are known to be potent broncho-constrictors [4] or to be responsible for vascular permeability changes those can lead to gastrointestinal (GI) damage. These adverse effects limit the use of anti-inflammatory agents for long duration.

Hence, in the present study, we evaluated the effect of YG on inhibition of COX-1, COX-2 and 5-LOX enzymes in vitro.

Materials and Methods

Study drugs

YG was procured in powder form from Shri Dhootpapeshwar Ltd. Aqueous extract was prepared by hot water extraction method and only water soluble portion was used for the study. The effect was studied at 3 concentrations viz., 25, 50 and 100µg/ml. This
concentration range was derived from the therapeutic dose of the formulation [5]. Aspirin was purchased from Sigma-Aldrich Chemicals Co., St. Louis Mi, USA and was used at 100 µg/ml corresponding to its anti-inflammatory human dose.

Chemicals and Kits

The inhibition of COX and LOX enzymes was assessed by Enzyme Immuno Assay (EIA) using standard kits purchased from M/s Cayman Chemicals. A standard curve was first plotted before evaluating the effect of the test formulation using five concentrations of the standards. A non-specific binding sample, a maximum binding sample, background samples and total activity sample was provided with the kits. Once the standard values were confirmed to be within the range stated by the manufacturer, percentage inhibition of the enzymes caused by the test formulation was determined by EIA. All the reaction procedures were performed as per the manufacturer’s instructions.

Statistical tests

All the results have been expressed as Mean± SD. Percent inhibition produced by YG for COX and LOX enzyme was calculated as per the formula provided by the manufacturer and the inhibition was compared with that obtained with aspirin. For inhibition of 5-LOX enzymes, ANOVA was used to compare the effect with aspirin followed by post hoc test using the Graph pad Instat software (version 3.06). A value of p<0.05 was considered as statistically significant.

Results

Inhibition of COX-1 enzyme

All the concentrations of YG inhibited the COX-1 enzyme to varied degrees [Table-1]. Maximum inhibition was obtained at 100µg/ml. However, the effect at all the concentrations was greater when compared to Aspirin.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration</th>
<th>% Inhibition of COX-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>100 µg/ml</td>
<td>36.98 ± 8.97</td>
</tr>
<tr>
<td>YG</td>
<td>25 µg/ml</td>
<td>53.62 ± 2.96</td>
</tr>
<tr>
<td></td>
<td>50 µg/ml</td>
<td>43.18 ± 1.24</td>
</tr>
<tr>
<td></td>
<td>100 µg/ml</td>
<td>58.29 ± 7.39</td>
</tr>
</tbody>
</table>

All values are indicative of Mean ± SD

Inhibition of COX-2 enzyme

YG showed a dose dependent inhibition of COX-2 enzyme with maximum effect seen at 100 µg/ml, which was comparable to the effect of aspirin [Table 2].

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration</th>
<th>% Inhibition of COX-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>100 µg/ml</td>
<td>59.02 ± 6.45</td>
</tr>
<tr>
<td>YG</td>
<td>25 µg/ml</td>
<td>35.74 ± 6.86</td>
</tr>
<tr>
<td></td>
<td>50 µg/ml</td>
<td>34.96 ± 0.91</td>
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<tr>
<td></td>
<td>100 µg/ml</td>
<td>58.36 ± 10.96</td>
</tr>
</tbody>
</table>

All values are indicative of Mean ± SD

Inhibition of 5-LOX enzyme

YG showed a dose dependant increase in percentage inhibition of 5-LOX enzyme [Table 3]. The activity exhibited by YG at all concentrations was significantly greater when compared with Aspirin.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration</th>
<th>% Inhibition of LOX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>100 µg/ml</td>
<td>15.7 ± 5.03</td>
</tr>
<tr>
<td>YG</td>
<td>25 µg/ml</td>
<td>31.4 ± 6.3**</td>
</tr>
<tr>
<td></td>
<td>50 µg/ml</td>
<td>35.4 ± 6.6***</td>
</tr>
<tr>
<td></td>
<td>100 µg/ml</td>
<td>43.26 ± 5.6***</td>
</tr>
</tbody>
</table>

All values are indicative of Mean ± SD

**p<0.01; ***p<0.001 as compared to Aspirin (100µg/ml) (ANOVA followed by post hoc test)

Discussion

In the present study, we evaluated the anti-inflammatory effect of YG by studying inhibition of two major enzymes involved in inflammatory cascade viz., COX and LOX. We found that YG demonstrated a substantial inhibition of 5-LOX enzyme which was greater than aspirin in addition to its inhibitory effect on COX-1 and COX-2 enzyme. These findings signify the dual effect of YG to inhibit COX and 5-LOX with a better anti-inflammatory potential as compared to selective COX inhibitors. Although NSAIDs are the drug of choice in treating inflammatory diseases, their main limitation is their side-effects, which includes gastrointestinal ulcerogenic activity and bronchospasm [6,7]. There has been some concern over the use of selective COX-2 inhibitors for therapeutic intervention, especially since some of these agents, like celecoxib and rofecoxib have been withdrawn by various countries including India due to their cardiovascular toxicity profile. 5-LOX inhibitors also represent an insufficient single therapeutic model in inflammatory diseases other than asthma. Thus, the discovery of compounds that can inhibit both the main metabolic pathways of the arachidonic acid metabolism is worthy of interest. The role of leukotrienes as inflammatory mediators has made them therapeutic targets, and many inhibitors aimed at leukotriene
biosynthetic or effector mechanisms are being developed. [8] In this context, YG can prove a potential agent. Further, lipooxygenase inhibitors like Zileuton have been shown to play an important role in inflammatory disorders of the respiratory such as bronchial asthma & COPD [9].
The classical shloka on YG in the Ayurvedic textbooks mentions that it can be used in the treatment of respiratory disorders. The LOX inhibitory activity of YG thus substantiates this claim. In our previous study Commiphora wightii, the major constituent of YG has shown significantly lower inhibition of 5-LOX enzyme as compared to Aspirin (data on file separately). Conversely YG demonstrated a significant inhibition of 5-LOX enzyme in the present study. This effect may be due to the synergistic activity exhibited by all the plants in the formulation of YG. However we did not compare the effect with a known LOX inhibitor like Zileuton which would have validated our results. Another important point is that we used the active water-soluble portion of the drug in the study while the formulation consists of plant extracts containing both water-soluble and insoluble components. The potential activity exhibited by YG may be due to the presence of actives present in the water soluble portion of the aqueous extract. Hence phyto-chemical investigations of whole extract vis-à-vis the water soluble portion followed by bioassay guided fractionation is desirable to confirm its activity. It would be interesting further to confirm these results using in vivo studies followed by clinical studies.

Conclusion

Our study demonstrates the role of YG as a potent anti-inflammatory agent as indicated by inhibition of COX-2 enzyme with comparatively reduced risk of GI side effects as indicated by its minimal inhibition of COX-1 enzyme. The 5-LOX inhibitory activity exhibited by YG provides a lead to explore its role further as a dual inhibitor of COX/5-LOX pathways and also to investigate its role as a potential agent to treat respiratory inflammatory disorders.

Acknowledgement

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References


