INTRODUCTION

Inflammation is a normal protective response to tissue injury and it involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair (Vane et al., 1995). It is a complex process, which is frequently associated with pain and involves occurrences such as increase in vascular permeability, increase of protein denaturation and membrane alterations (Umapathy et al., 2010). Harmful stimuli including pathogens, irritants or damaged cells initiate response of vascular tissue as inflammation. Inflammation is a protective attempt by the organism to remove injurious stimuli as well as initiation of the healing process for the tissue (Denko, 1992). However, if inflammation is not treated it leads to onset of diseases like vasomotor rhinorrhea, rheumatoid arthritis and atherosclerosis (Henson et al., 1989). In appreciating the inflammatory process, it is important to understand the role of chemical mediators. These are substances that tend to direct the inflammatory response. These inflammatory mediators come from plasma proteins or cells including mast cells, platelets, neutrophils and monocytes. They are triggered by bacterial products or host proteins. Chemical mediators bind to specific receptors. Vascular permeability, neutrophil chemotaxis, stimulate smooth muscle contraction, have direct enzymatic activity which induce pain or mediate oxidative damage. Most mediators are short - lived but cause harmful
effects. Examples of chemical mediators include vasoactive amines (histamine, serotonin), arachidonic acids (prostaglandins, leukotrienes) and cytokines (tumour necrosis factor and interleukin-1) Smith et al., (2004). It is believed that current drugs available such as opioids and non-steroidal anti-inflammatory drugs (NSAIDS) are not useful in all cases of inflammatory disorders, because of their side effects and potency (Ahmadiani et al., 1998). As a result, a search for other alternatives seems necessary and beneficial. Hence the study of plants that have been used traditionally for curing inflammation needs to be analysed.

The polyherbal formulation Amukkara chooranam\textsuperscript{[1]} is analysed for its anti-inflammatory activity which may act by inhibiting prostaglandins of cyclo-oxygenase pathway.

**OBJECTIVE**

The aim of the present study is to evaluate the in vitro assay Anti-inflammatory activity of Amukkara chooranam.

**MATERIALS AND METHODS**

**Albumin Denaturation Assay Procedure**

In-vitro anti-inflammatory activity Amukkara chooranam was studied using albumin denaturation technique. The reaction mixture consisted of bovine serum albumin (5% aqueous solution) and test sample Amukkara chooranam at varying concentration ranges from 100 to 500 µg/ml and standard Diclofenac sodium at the concentration of 100 µg/ml of final volume (Figure 2). pH was adjusted by using a small amount of 1N Hydrochloric acid. The samples were incubated at 37°C for 20 min and then heated at 57°C for 3 min. After cooling the sample, 2.5 ml of phosphate buffer solution was added into each test tube. Turbidity developed was measured spectrophotometrically at 660 nm, for control distilled water was used instead of test sample while product control tests lacked bovine serum albumin. The experiment was performed in triplicate.

The Percentage protection from denaturation is calculated by using the formulae

\[
\left[ \frac{(A)_{\text{control}} - (A)_{\text{sample}}}{(A)_{\text{control}}} \right] \times 100. 
\]
RESULTS AND OBSERVATION

The result obtained from the present study clearly indicates that the test drug Amukkara Chooranam was effective in inhibiting heat induced albumin denaturation technique.
Maximum percentage inhibition of about 70.89% was observed at 500 μg/ml when compared to that of the Diclofenac sodium, a standard anti-inflammatory agent with the maximum inhibition 94.74% at the concentration of 100 μg/ml.

Table 1: Final Result.

<table>
<thead>
<tr>
<th>Concentration in µg/ml</th>
<th>Percentage Inhibition of Protein Denaturation</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC 100</td>
<td>17.02 ± 7.30</td>
</tr>
<tr>
<td>AC 200</td>
<td>32.06 ± 4.26</td>
</tr>
<tr>
<td>AC 300</td>
<td>38.34 ± 2.95</td>
</tr>
<tr>
<td>AC 400</td>
<td>51.52 ± 0.83</td>
</tr>
<tr>
<td>AC 500</td>
<td>70.89 ± 5.28</td>
</tr>
<tr>
<td>Diclofenac sodium (100 µg)</td>
<td>94.74 ± 4.25</td>
</tr>
</tbody>
</table>

Note: Each value represents the mean ± SD. N=3

Fig. 5: In vitro Anti-inflammatory activity of Amukkara chooranam.
RESULT ANALYSIS
The result obtained from the present clearly indicates that the test drug Amukkara chooranam was effective in inhibiting heat induced albumin denaturation. Maximum percentage inhibition of about 70.89% was observed at 500 μg/ml when compare to that of the Diclofenac sodium, a standard anti-inflammatory agent with the maximum inhibition 94.74% at the concentration of 100 μg/ml. (Table 1) (Fig 5).

CONCLUSION
From the result of the study it was concluded that the test drug Amukkara chooranam possess significant anti-inflammatory property in protein denaturation assay.

REFERENCES
7. Statistical analysis: Results are expressed as Mean ± SD. The difference between experimental groups was compared by One-Way Analysis Of Variance (ANOVA) followed by Dunnet Multiple comparison test.