A COMPARATIVE STUDY TO EVALUATE ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY OF COMMONLY USED PROTEOLYTIC ENZYMES AND THEIR COMBINATION WITH DICLOFENAC IN RATS

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ABSTRACT: Introduction: Proteolytic enzymes like serratiopeptidase, trypsin and chymotrypsin are used for prolonged period for edema resolution in post-operative patients, arthritis, tooth extraction etc. either alone or with other anti-inflammatory agent. Various trials have supported and refused the role of enzymes as anti-inflammatory agents. No study was found for analgesic property of proteolytic enzymes. Aims: This study was designed to investigate the anti-inflammatory and analgesic activity of these three proteolytic enzymes alone and in combination with diclofenac at therapeutic doses.

Methods and Material: Carrageenan induced paw edema was used for acute inflammation. Edema was measured with the help of plethysmometer at 0 hour and 3 hour. Modified formalin test was used to evaluate the analgesic property. 1% formalin was injected into the forepaw and scoring was done by appropriate and standard method. Statistical Analysis: Edema changes of hind paw and licking duration in seconds of a fore paw with different treatments were compared with one-way ANOVA followed by Tukey-Kramer multiple comparison test with the help of graph pad instat software. Result: Reduction in edema was significant (p<0.05) with proteolytic enzymes alone as well as in combination with diclofenac. Neither phase of formalin test was reduced significantly by proteolytic enzymes alone although second phase was reduced significantly when combined with diclofenac. Conclusion: Serratiopeptidase, trypsin and chymotrypsin has significant anti-inflammatory activity alone as well as in combination with diclofenac. These proteolytic enzymes do not have any analgesic activity but they potentiate the peripheral analgesic activity of diclofenac at therapeutic equivalent doses.

INTRODUCTION: Inflammation is nature’s double edged sword. Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used to control pain and inflammation but they also have side effects 1, 3. Proteolytic enzymes are used commonly for their anti-inflammatory property. Various clinical trials have confirmed the role of serratiopeptidase as anti-inflammatory agent. Serratiopeptidase and chymotrypsin is used to control inflammation in airway diseases, osteoarthritis, carpal tunnel syndrome, dental extraction and cataract surgery alone and in combination with other agents 4, 9. These proteolytic enzymes are commonly used in combination with conventional NSAIDs like diclofenac. Serratiopeptidase, trypsin and chymotrypsin are studied alone as well as in combination with NSAIDs for their anti-inflammatory property but not for their analgesic property. So this study was primarily aimed to...
evaluate whether these proteolytic enzymes have anti-inflammatory and analgesic property alone and synergistic activity with diclofenac at therapeutic doses as claimed?

MATERIALS AND METHODS:
The study protocol was approved by institutional animal ethics committee. Wistar albino rats of either sex weighing 200-300gms were used for the study. Animals were housed in a room with the temperature at 25±1°C with alternating 12 hour light dark cycle. They were subjected to standard diet and water ad libitum.

Trypsin and chymotrypsin, Serratiopeptidase and Diclofenac were used in the study.

The therapeutic doses of all the drugs were converted to rat equivalent with the help of conversion table 10, 11. All the drugs were dissolved in normal saline. To study anti-inflammatory and analgesic activity rats were divided into fourteen groups, seven for both property and each group containing six rats. All the treatments were given orally with the help of the rat gavage canula. As trypsin and chymotrypsin are available in combination in market, here also they were used in combination.

Group 1: Control group, 0.3ml normal saline.

Group 2: Diclofenac-15mg/kg.

Group 3: Serratiopeptidase-3mg/kg.

Group 4: Trypsin-5.76 mg/kg.

Group 5: Chymotrypsin-36mg/kg.

Group 6: Diclofenac-15mg/kg with Serratiopeptidase-3mg/kg.

Group 7: Diclofenac-15mg/kg with Trypsin-5.76mg/kg and Chymotrypsin-36mg/kg.

Anti-inflammatory activity:
To study anti-inflammatory activity, carrageenan induced rat hind paw edema model was used 12. 0.1ml of 1% λ-Carrageenan made into normal saline was injected into subplanter space in the right hind paw of all rats. The rats were marked with black ink at right lateral malleolus. Drugs dissolved in normal saline were given half an hour before the injection of carrageenan. Paw volumes of all the rats were measured before giving an injection of carrageenan which was considered as 0 minute reading. The second reading was taken at 3 hours. Percentage inhibition was calculated by using the following formula 13:

Eqn. 1: Percent swelling = (V-Vi/Vi) ×100, where V is the paw volume 3 h after the carrageenan injection, and Vi is the initial paw volume. The average paw swelling in the group of the drug-treated rats were compared with control rats and the percent of inhibition of the edema formation was determined using,

Eqn. 2: Percent inhibition = [1-percent swelling of drug treated group/percent swelling of control group] ×100.

Analgesic activity:
Modified formalin test in rats was used to study analgesic activity 14. Animals show two phases (biphasic) of nociceptive behavior involving two different stimuli when formalin is injected. The first phase starts immediately after injection of formalin and lasts for 3-5minutes. This occurs due to chemical stimulation of nociceptors causing C-fiber activation. Second late phase starts after 15minutes of formalin injection and last for 20-40minutes. This phase appears due to combination of an inflammatory reaction in the peripheral tissue 15.

Pain intensity was rated according to following numerical scale 16.

T0 - Both fore paws are placed on the floor and weight is evenly distributed.

T1 - The injection paw rests lightly on the floor or on another part of the animal’s body and little or no weight is placed upon it.

T2 - The injected paw is elevated and not in contact with any surface. The uninjected paw is placed firmly on the floor.
The injected paw is licked, bitten or shaken, while the uninjected paw is not.

To study analgesic activity 0.05 ml of 1% formalin was injected in right front paw of rat subcutaneously. Seven groups were used for this study. Rats were given above described treatments thirty minutes before injecting formalin.

The rats were observed for 60 minutes after the injection of formalin and the amount of time (second) spent in each scale (0, 1, 2 and 3) was recorded. Ratings are averaged over five minute block. Numerical ratings are calculated from the following formula:

Eqn 3: Pain rating = T1+2T2+3T3
300

Where T1, T2 and T3 are durations in seconds spent in each five minute block.

Statistical analysis:

TABLE 1: COMPARISON OF PAW EDEMA AT 3 HOUR.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Treatment</th>
<th>Dose Mg/kg</th>
<th>Edema(ml) Mean±SEM</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (Normal saline)</td>
<td>0.3ml</td>
<td>1.58±0.02</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Diclofenac</td>
<td>15</td>
<td>1.19±0.03</td>
<td>46%*</td>
</tr>
<tr>
<td>3</td>
<td>Serratiopeptidase</td>
<td>3</td>
<td>1.36±0.02</td>
<td>35%*</td>
</tr>
<tr>
<td>4</td>
<td>Trypsin</td>
<td>5.76</td>
<td>1.42±0.01</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Chymotrypsin</td>
<td>36</td>
<td>1.37±0.02</td>
<td>40%*</td>
</tr>
<tr>
<td>6</td>
<td>Diclofenac+Serratiopeptidase</td>
<td>15 + 3</td>
<td>1.05±0.05</td>
<td>60%*#</td>
</tr>
<tr>
<td>7</td>
<td>Diclofenac+Trypsin+Chymotrypsin</td>
<td>15 + 5.76 + 36</td>
<td>1.02±0.01</td>
<td>64%*#</td>
</tr>
</tbody>
</table>

n=6 in each group
* p<0.05 (one way-ANOVA and Tukey-Kramer multiple comparison test),
* - significant compared to the control
# - significant compared to Diclofenac

Edema changes of the hind paw (anti-inflammatory activity) and licking duration in seconds (analgesic activity) of a fore paw with different treatments were compared with one-way ANOVA followed by Tukey-Kramer multiple comparison test with the help of graph pad instat software.

RESULTS: In acute inflammation model carrageenan induced paw edema was significantly (p<0.05) reduced by all proteolytic enzymes compared to control. Table 1 shows the percentage inhibition of edema by serratiopeptidase, trypsin and chymotrypsin was 44%, 35% and 40% respectively. The percentage inhibition of edema by diclofenac was 46%. Serratiopeptidase when combined with diclofenac shows inhibition of 60% which is significant to both control and diclofenac alone also. Combination of Trypsin and chymotrypsin when used with diclofenac shows inhibition of 64% which is significant compared to control and diclofenac.
In modified formalin test, biphasic responses were seen. Fig.1 shows proteolytic enzymes reduced the second phase of the formalin test but not the first phase although reduction of the second phase was not significant. Diclofenac inhibited the second phase significantly but it was not effective in reducing the first phase. The addition of proteolytic enzymes to diclofenac resulted in further inhibition of the second phase while phase 1 remains unaffected. This inhibition is significant compared to control and diclofenac alone.

DISCUSSION: Proteolytic enzymes have been promoted by the pharmaceutical companies since long time and they are also being used by the medical community. Diclofenac like agents are principally used to control pain and inflammation in various inflammatory conditions. Proteolytic enzymes are used either alone or in combination with other anti-inflammatory agents. Few studies have supported the role of proteolytic enzymes as anti-inflammatory agents while few have made doubt. Swamy et al (2008) has shown that proteolytic enzymes have anti-inflammatory activity and exhibits synergistic effect with aspirin in both acute and subacute models of inflammation in rats.

The findings of the present study indicate that serratiopeptidase, trypsin and chymotrypsin have suppressed the inflammation significantly. The probable mechanisms of anti-inflammatory activity are neutrophils apoptosis, inhibition of neutrophils migration at inflammatory site, inhibition of bradykinin synthesis, and clearing inflammatory debris. Combining these proteolytic enzymes with diclofenac gives additional anti-inflammatory activity which means these enzymes may have potentiated the anti-inflammatory activity of diclofenac.

Analgesic property of these proteolytic enzymes has not been explored. The modified formalin test was used because it allows even weak analgesics to be detected. It shows phase-1 and phase-2 due to stimulation of nerve endings and inflammation respectively. Analgesics like opioids are effective in inhibiting the phase-1 while NSAIDs like analgesics are effective in inhibiting phase-2. Results of analgesic property by modified formalin test shows that they are not effective analgesics as neither phase of biphasic response were suppressed significantly except slight lowering of second phase. This suggests that they have poor peripheral analgesic action. The mechanism may be inhibition of bradykinin synthesis which may interfere with sensitization of peripheral nerve endings which leads to mild peripheral analgesic action. Addition of these proteolytic enzymes to diclofenac leads to greater analgesia as their analgesic action adds up.

CONCLUSION: The observation of the present study indicates that serratiopeptidase, trypsin and chymotrypsin have anti-inflammatory activity at therapeutic doses but they are not effective as analgesic. Combination of these proteolytic enzymes with diclofenac should be preferred over only proteolytic enzymes as greater anti-inflammatory and analgesic action is achieved.

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CONFLICT OF INTEREST: None

REFERENCES: