CONTENTS

REVIEW ARTICLES
A Decision Tree for Rapid Quality Assurance and Control of
Rifampicin-Containing Oral Dosage Forms for Global
Distribution for Tuberculosis Treatment
Y. ASHOKRAJ, SHRUTIDEVI AGRAWAL AND R. PANCHAGNULA
Transdermal Delivery by Iontophoresis
SWATI RAWAT, SUDHA VENUGURLEKAR, B. RAKESH,
S. JAIN, G. SRIKARTI

RESEARCH PAPERS
In vivo Evaluation of Single Dose Tetanus Toxoid Vaccine
Formulation with Chitosan Microspheres
R. MANIVANNAN, S. A. DHANARAJ, Y. UDAYA BHASKARA RAO, A. BALASUBRAMANIAM, N. L. GOWRISHANKAR,
N. JAWAHAR AND S. JUBIE
Ionic Cross-linked Chitosan Beads for Extended Release of Ciprofloxacin: In vitro Characterization
A. SRINATHA, J. K. PANDIT AND S. SINGH
Design and Optimization of Diclofenac Sodium
Controlled Release Solid Dispersions by Response Surface Methodology
H. N. SHIVAKUMAR, B. G. DESAI AND G. DESHMUKH
Evaluation of Free Radical Scavenging Activity of an Ayurvedic Formulation, Panchvalkala
SHEETAL ANANDJIWALA, M. S. BAGUL, R. MANIVANNAN, B. M. PATEL, S. JAIN AND M. RAJANI
Validation of Different Methods of Preparation of Adhatoda vasica Leaf Juice by Quantification of Total Alkaloids and Vasicine
S. SONI, SHEETAL ANANDJIWALA, G. PATEL AND M. RAJANI
Formulation and Characterization of Mucoadhesive Buccal Films of Glipizide
MONA SEMALTY, A. SENALTY AND G. KUMAR
Synthesis, Antimicrobial and Anti-inflammatory Activity of 2,5-Disubstitued-1,3,4-oxadiazoles
G. NAGALAKSHMI
Ascorbic Acid Inhibits Development of Tolerance and Dependence to Opiates in Mice: Possible Glutamatergic or Dopaminergic Modulation
S. K. KULKARNI, C. DESHPANDE AND A. DHIR
Design and In vitro Characterization of Buccoadhesive Drug Delivery System of Insulin
J. SAHNI, S. RAJ, F. J. AHMAD AND R. K. KHAR
Development and Evaluation of a Choramphenicol Hypertonic Ophthalmic Solution
A. V. JITHAN, C. KRISHNA MOHAN, AND M. VIMALADEVI
Optimization of Fast Dissolving Etoricoxib Tablets Prepared by Sublimation Technique
D. M. PATEL AND M. M. PATEL
Furosemide-loaded Alginate Microspheres Prepared by Ionic Cross-linking Technique: Morphology and Release Characteristics
M. K. DAS AND P. C. SENAPATI

SHORT COMMUNICATIONS
Isolation of Liver Aldehyde Oxidase Containing Fractions from Different Animals and Determination of Kinetic Parameters for Benzaldehyde
R. S. KADAM AND K. R. IVER
Microwave-Induced Synthesis of Schiff Bases of Aminothiazolyl Bromocoumarins as Antibacterials
K. N. VENUGOPALA AND B. S. JAYASHREE
In vitro Antiviral Activity of some Novel Isatin Derivatives against HCV and SARS-CoV Viruses
P. SELVAM, N. MURUGESH, M. CHANDRAMOHAN, E. DE CLERCQ, E. KEYAERTS, L. VIJGEN, P. MAES,
J. NEYTS AND M. V. RANST

PHYSICOCHEMICAL AND PHARMACOKINETIC PARAMETERS IN DRUG SELECTION AND LOADING FOR TRANSDERMAL DRUG DELIVERY
N. S. CHANDRASHEKAR AND R. H. SHOBHA RANI
HPLC Estimation of berberine in Tinospora cordifolia and Tinospora sinensis
G. V. SRINIVASAN, K. P. UNNIKRISHNAN, A. B. REMA SHREE AND INDIRA BALACHANDRAN
Parenteral Formulation of Zopiclone
P. V. SWAMY, P. SUSHMA, G. CHIRAG, K. PRASAD, M. YOUNUS ALI AND S. A. RAJU

Simultaneous Spectrophotometric Determination of Lansoprazole and Domperidone in Capsule Dosage Form
A. P. SHERUE, A. V. KASTURE, K. N. GUJAR AND P. G. YEOLE
Novel 2-Pyrazoline Derivatives as Potential Antibacterial and Antifungal Agents
SUVARNA KINI AND A. M. GANDHI

Spectrophotometric Determination of Ethamsylate and Mefenamic Acid from a Binary Mixture by Dual Wavelength and Simultaneous Equation Methods
ANJU GOYAL AND I. SINGHV
Novel Colon Targeted Drug Delivery System Using Natural Polymers
V. RAVI, T. M. PRAMOD KUMAR AND SIDDARAMAIAH

Effect of Some Clinically Used Proteolytic Enzymes on Inflammation in Rats
A. H. M. VISWANATHA SWAMY AND P. A. PATIL

Synthesis and Pharmacological Evaluation of (6-Substituted 4-Oxo-4H-chromene-3 yl) methyl N-substituted Aminoacetates
ASMITA GAJBHIYE, V. MALLAREDDY AND G. ACHAIH

Development and In vitro Evaluation of Buccoadhesive Tablets of Metoprolol Tartrate
P. D. NAKHAT, A. A. KONDAWAR, L. G. RATHI AND P. G. YEOLE

RP-HPLC Estimation of Venlafaxine Hydrochloride in Tablet Dosage Forms
S. I. BALDANIA, K. K. BHATT, R. S. MEHTA, D. A. SHAH AND INDIRA BALACHANDRAN

Simultaneous Estimation of Esomeprazole and Domperidone by UV Spectrophotometric Method
S. LAL, S. S. PRABHU, A. SHIRWAIKAR, ANNI SHIRWAIKAR, C. DINESH KUMAR, A. JOSEPH AND R. KUMAR

In vitro Anthelmintic Activity of Baliospermum montanum Muell. Arg roots
R. G. MALI AND R. R. WADEKAR

REFERENCES FOR INDIAN JOURNAL OF PHARMACEUTICAL SCIENCES DURING 2006 & 2007

[85-88]
[89-91]
[91-94]
[94-96]
[102-105]
[105-108]
[108-111]
[111-113]
[114-117]
[118-120]
[114-117]
[118-120]
[121-124]
[124-128]
[128-131]
[131-133]
[134-134]
Effect of Some Clinically Used Proteolytic Enzymes on Inflammation in Rats

A. H. M. VISWANATHA SWAMY* AND P A. PATIL1
1Department of Pharmacology and Pharmacotherapeutics, J. N. Medical College, Nehru Nagar, Belgaum - 580 010, India

Swamy, et al.: Effects of Proteolytic Enzymes on Inflammation in Rats

The study was designed to investigate the role of three proteolytic enzymes viz., chymotrypsin, trypsin and serratiopeptidase on hind paw edema and cotton pellet induced granuloma and their possible interactions with aspirin in albino rats. Animals were treated with proteolytic enzymes alone in three different doses or aspirin or in combination with subantiinflammatory dose of aspirin or saline, 30 min before injection of 0.1 ml 1% carrageenan. Paw volume was measured before and 3 h after the injection of carrageenan. Chymotrypsin, (5, 18 and 36 mg/kg), trypsin (1.44, 2.88 and 5.76 mg/kg) and serratiopeptidase (0.45, 0.9 and 2.70 mg/kg) were showed dose dependent antiinflammatory activity in acute model of inflammation. Serratiopeptidase showed better antiinflammatory activity on carrageenan induced inflammation than other two proteolytic enzymes and aspirin. However, chymotrypsin and serratiopeptidase were found to be more effective than aspirin in subacute model of inflammation. Chymotrypsin, trypsin and serratiopeptidase possess antiinflammatory activity and exhibit synergistic effect with aspirin in both acute and subacute models of inflammation in rats.

Key words: Inflammation, proteolytic enzymes, aspirin

Inflammation is a normal response to protect the tissues from various noxious stimuli and is one of the most normal clinical conditions. A wide variety of enzymes and enzyme mixtures have been used as adjunctive therapeutic agents in a number of clinical conditions particularly in trauma and orthopedic clinics. Proteolytic enzymes are co-administered with non-steroidal antiinflammatory agents. Based on the earlier reports, it is suggested that the presence of proteolytic enzymes like chymotrypsin, cathepsin D1 and other proteases2 in inflammatory exudates indicate their role in the process of inflammation3,5. On the other hand, proteolytic activities of these enzymes have been proposed to be vital for the control of inflammation by clearing inflammatory debris6,7. There are several reports advocating the use of these proteolytic enzymes for the treatment of inflammatory disorders8,9. While other reports indicate that despite extensive clinical experience their antiinflammatory activity is unproved5 and controversial8. The literature survey indicates that there is paucity of information regarding serratiopeptidase and interaction with NSAID's. As controversies about

the role of proteolytic enzymes like chymotrypsin, trypsin and serratiopeptidase on inflammation still exists, the present study was undertaken to probe antiinflammatory activity of these enzymes and their possible interactions with aspirin.

Wistar rats of either sex weighing between 120 and 150 g were used for the study. Animals were housed in a room temperature maintained at 22±1°C with an alternating 12 h light-dark cycle. They were subjected to standard diet and water ad libitum. Trypsin, purchased from S. D. Fine Chemicals, Mumbai and aspirin was procured form Swastik Pharmaceuticals, Mumbai. Chymotrypsin and serratiopeptidase were purchased from the local market as alfapsin and bidanzen respectively. All the animal experimental protocol has been approved by the institutional animal ethics committee.

The clinical doses of all the three proteolytic enzymes used were converted to rat equivalents with the help of conversion Table10. All the drugs were dissolved in saline and were administered orally half an hour prior to the carrageenan injection and thereafter repeated once daily for 10 days in subacute model of inflammation.

Acute inflammation was produced by subplantar injection of 0.1 ml of 1% suspension of carrageenan in normal saline in the right hind paw of the rats11. Measurement of paw volume was done using

*For correspondence
E-mail: vmhiremath2004@yahoo.com
Department of Pharmacology,
KLES College of Pharmacy,
Vidyanagar, Hubli - 580 031, India.
Table 1: Effect of proteolytic enzymes and standard drug on carrageenan induced paw edema

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Edema at 3rd h</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Normal saline</td>
<td>1.08 ± 0.03</td>
<td>56.09*</td>
</tr>
<tr>
<td>Aspirin</td>
<td>200.00</td>
<td>0.18 ± 0.03</td>
<td>92.5</td>
</tr>
<tr>
<td>Chymotrypsin</td>
<td>9.00</td>
<td>0.98 ± 0.24</td>
<td>32.40*</td>
</tr>
<tr>
<td>Chymotrypsin</td>
<td>18.00</td>
<td>0.73 ± 0.12</td>
<td>49.07*</td>
</tr>
<tr>
<td>Chymotrypsin</td>
<td>36.00</td>
<td>0.41 ± 0.06</td>
<td>62.81*</td>
</tr>
<tr>
<td>Trypsin</td>
<td>1.44</td>
<td>0.83 ± 0.11</td>
<td>23.14</td>
</tr>
<tr>
<td>Trypsin</td>
<td>2.88</td>
<td>0.70 ± 0.09</td>
<td>35.18*</td>
</tr>
<tr>
<td>Traspin</td>
<td>5.76</td>
<td>0.55 ± 0.05</td>
<td>43.51*</td>
</tr>
<tr>
<td>Traspin + Aspirin</td>
<td>0.45</td>
<td>1.03 ± 0.04</td>
<td>60.18*</td>
</tr>
<tr>
<td>Serratiopeptidase</td>
<td>0.90</td>
<td>0.47 ± 0.18</td>
<td>62.81*</td>
</tr>
<tr>
<td>Serratiopeptidase</td>
<td>2.70</td>
<td>0.38 ± 0.05</td>
<td>43.51*</td>
</tr>
<tr>
<td>Chymotrypsin + Aspirin</td>
<td>9.00 + 54.00</td>
<td>0.43 ± 0.04</td>
<td>60.18*</td>
</tr>
<tr>
<td>Trypsin + Aspirin</td>
<td>1.44 + 54.00</td>
<td>0.61 ± 0.04</td>
<td>60.97*</td>
</tr>
<tr>
<td>Serratiopeptidase + Aspirin</td>
<td>0.45 + 54.00</td>
<td>0.16 ± 0.02</td>
<td>60.97*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM; n = 6 animals in each group; *p < 0.05 when compared to control.
TABLE 4: EFFECT OF PROTEOLYTIC ENZYMES AND STANDARD DRUG ON WEIGHT OF ADRENAL GLANDS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Mean weight (mg%BW)</th>
<th>% Increases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Normal Saline</td>
<td>9.58 ± 0.767</td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>200.00</td>
<td>27.66 ± 2.60</td>
<td>39.24*</td>
</tr>
<tr>
<td>Chymotrypsin</td>
<td>18.00</td>
<td>26.46 ± 2.88</td>
<td>41.88*</td>
</tr>
<tr>
<td>Trypsin</td>
<td>2.88</td>
<td>29.22 ± 2.43</td>
<td>35.85*</td>
</tr>
<tr>
<td>Serratiopeptidase</td>
<td>0.90</td>
<td>25.15 ± 1.13</td>
<td>44.77*</td>
</tr>
<tr>
<td>Chymotrypsin + Aspirin</td>
<td>9.00 + 54.00</td>
<td>28.88 ± 1.82</td>
<td>36.58*</td>
</tr>
<tr>
<td>Trypsin + Aspirin</td>
<td>1.44 + 54.00</td>
<td>30.14 ± 0.58</td>
<td>33.00*</td>
</tr>
<tr>
<td>Serratiopeptidase + Aspirin</td>
<td>0.45 + 54.00</td>
<td>28.10 ± 4.23</td>
<td>38.28*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM; n = 6 animals in each group; *p < 0.05 when compared to control. †: % decrease and serratiopeptidase showed 39.97% and 89.94%, respectively. But in aspirin treated group the adrenal gland weight was decreased significantly as compared to control (Table 4).

Findings of the present study clearly indicate that chymotrypsin, trypsin and serratiopeptidase have suppressed inflammation significantly both in carrageenan as well as cotton pellet induced granuloma and appear to be dose dependent. The lowest dose of all the three enzymes and aspirin individually has failed to show any significant antiinflammatory activity on carrageenan induced inflammation, but in combination of all the above have potentiated antiinflammatory activity of aspirin. The potentiated antiinflammatory activity of aspirin was comparable to that of aspirin 200 mg/kg, not only in carrageenan induced but also in cotton pellet induced granuloma study. The antiinflammatory activity of these enzymes in both models of inflammation may be attributed due to stimulation of neutrophil apoptosis16, inhibition of neutrophil migration at the inflammatory site17, inhibition of bradykinin synthesis, decreased vascular permeability and by clearing inflammatory debris18-20.

Aspirin has expected to produce significant increase in the ulcer index as compared to control. While chymotrypsin, trypsin and serratiopeptidase alone and in combination with aspirin reduced ulcer index significantly as compared to aspirin treated animals. The reduction in ulcer index observed in this study may be due to boost of the defensive factors.

Adrenal gland weights of the animals treated with all the three proteolytic enzymes were significantly increased as compared to control but in contrast to aspirin treated animals in which it decreased

TABLE 2: EFFECT OF PROTEOLYTIC ENZYMES AND STANDARD DRUG ON COTTON PELLET INDUCED GRANULOMA FORMATION

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Mean granuloma wt (mg/100 g)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Normal Saline</td>
<td>45.54 ± 1.19</td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>200.00</td>
<td>27.66 ± 2.60</td>
<td>39.24*</td>
</tr>
<tr>
<td>Chymotrypsin</td>
<td>18.00</td>
<td>26.46 ± 2.88</td>
<td>41.88*</td>
</tr>
<tr>
<td>Trypsin</td>
<td>2.88</td>
<td>29.22 ± 2.43</td>
<td>35.85*</td>
</tr>
<tr>
<td>Serratiopeptidase</td>
<td>0.90</td>
<td>25.15 ± 1.13</td>
<td>44.77*</td>
</tr>
<tr>
<td>Chymotrypsin + Aspirin</td>
<td>9.00 + 54.00</td>
<td>28.88 ± 1.82</td>
<td>36.58*</td>
</tr>
<tr>
<td>Trypsin + Aspirin</td>
<td>1.44 + 54.00</td>
<td>30.14 ± 0.58</td>
<td>33.00*</td>
</tr>
<tr>
<td>Serratiopeptidase + Aspirin</td>
<td>0.45 + 54.00</td>
<td>28.10 ± 4.23</td>
<td>38.28*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM; n = 6 animals in each group; *p < 0.05 when compared to control.

TABLE 3: EFFECT OF PROTEOLYTIC ENZYMES AND STANDARD DRUG ON GASTRIC MUCOSA (ULCER INDEX)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Mean ulcer index ± SEM</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Normal Saline</td>
<td>10.00 ± 6.32</td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>200.00</td>
<td>40.00 ± 0.00</td>
<td></td>
</tr>
<tr>
<td>Chymotrypsin</td>
<td>18.00</td>
<td>5.00 ± 0.60</td>
<td>50.00*</td>
</tr>
<tr>
<td>Trypsin</td>
<td>2.88</td>
<td>5.60 ± 0.66</td>
<td>44.00*</td>
</tr>
<tr>
<td>Serratiopeptidase</td>
<td>0.90</td>
<td>4.21 ± 0.26</td>
<td>57.90*</td>
</tr>
<tr>
<td>Chymotrypsin + Aspirin</td>
<td>9.00 + 54.00</td>
<td>26.42 ± 5.83</td>
<td>33.95*</td>
</tr>
<tr>
<td>Trypsin + Aspirin</td>
<td>1.44 + 54.00</td>
<td>30.00 ± 4.21</td>
<td>25.00*</td>
</tr>
<tr>
<td>Serratiopeptidase + Aspirin</td>
<td>0.45 + 54.00</td>
<td>23.33 ± 7.602</td>
<td>41.67*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM; n = 6 animals in each group; *p < 0.05 when compared to control and #p < 0.05 when compared to aspirin.
significantly as compared to control. The reduction in the weight of adrenal glands in aspirin treated group may be because of production of corticosteroids which may in turn explain the antiinflammatory activity of aspirin\textsuperscript{21}. Increase in adrenal glands weight of proteolytic enzymes treated groups may indicate the release of catecholamines that are responsible for antiinflammatory activity\textsuperscript{22,23}. Further investigations are needed to establish these facts by biochemical estimations of corticosteroids and catecholamines in plasma.

The observation of the present study clearly indicates that antiinflammatory activity of all the three enzymes were dose dependent. The combination of low doses of these enzymes with sub antiinflammatory dose of aspirin resulted in synergistic antiinflammatory activity without ulcerogenic potential appears to be clinically a beneficial interaction. If the present findings could be extrapolated to human beings, such combination therapies may reduce the adverse effects of NSAID’s like aspirin. However clinical trials are further needed to be studied to confirm the same.

**ACKNOWLEDGEMENTS**

The authors are thankful to Dr. F. V. Manvi, Principal, K. L. E. S. College of Pharmacy, Belgaum, for his constant support during the present research.

**REFERENCES**