1. INTRODUCTION

Enzyme is a biocatalyst which accelerates biochemical reactions. The sources of enzymes are microorganisms, higher plants and animals. Microbial enzymes have gained much popularity. There are of two enzymes, extracellular enzymes and intracellular enzymes. The former is secreted outside the cell and later remain within the cell. Microbial enzymes have many advantages over the animal and plant enzyme, firstly; they are economical and can be produced on large scale within the limited space and time. Secondly, they are capable of producing a wide variety of enzymes; they can grow in a wide range of environmental conditions. They show genetic flexibility and so can be genetically manipulated to increase the yield of enzymes.

Enzymes have many roles in the pharmaceutical and diagnostic industries. There are too many applications to cover but typical applications include:

- Enzymes are direct pharmaceutical products such as in the treatment of genetic disorders leading to a specific enzyme deficiency.
- Extraction of medicinally important compounds such as heparin.
- Combinational biocatalysts.
- Manufacture of pharmaceuticals chemically where enzymes are used for inter conversion of chemical intermediates and removal of unwanted products (Anilkumar and Sadhana Nighojkar, 2005).
Enzymes are widely used in agriculture, animal feeds, baking, brewing, detergent industry, starch industry, pharmaceuticals and diagnostics.

Serratiopeptidase, also known as Serrapeptase or Serratia peptidase, is a proteolytic enzyme isolated from the non pathogenic enterobacteria Serratia marcescens. This enzyme is found naturally in the intestine of the silkworm, which is used by the silkworm to dissolve the cocoon and emerge as a moth. Serratiopeptidase is a proteolytic enzyme isolated from the micro-organism Serratia marcescens. This was discovered in the silkworm intestine (Noboru Matsumoto et al., 1984). Histological studies reveal powerful anti-inflammatory effects of this naturally occurring enzyme. Serratiopeptidase is a naturally occurring, physiological agent with no inhibitory effects on prostaglandins and is devoid of gastrointestinal side effects. Serratiopeptidase dissolves blood clots. It is used to treat cardiovascular diseases, Arthritis, Eye problems, etc. The search for a physiological agent that offers anti-inflammatory properties without causing side effects ended with discovery of serratiopeptidase. It does not interfere with the synthesis of cholesterol in the body.

Serratia marcescens is a gram negative, bacillus shaped bacteria that belongs to the family Enterobacteriaceae. In 1919, Bartolomeo Bizio, an Italian pharmacist from Padua, discovered S.marcescens. He identified the bacterium as the cause of the miraculous bloody discoloration of cornmeal mush, or polenta. He named Serratia in honour of the Italian physicist Serratia who invented the steamboat, and named marcescens from the Latin word for decaying, because the bloody coloration quickly disappeared. Key characteristics of S. marcescens include the production of DNase, lipase and gelatinase and it is oxidase
negative. In early to mid 1900’s *S. marcescens* was frequently used by physicians as a biological marker in studying transmission of microorganisms because it was believed to be harmless. In fact, teachers and scientists commonly need *Serratia marcescens* in experiments of microbial transmission and to demonstrate the importance of hand washing.

In 1951 and 1952, the US army conducted the operation sea spray with *Serratia marcescens*. It colonizes in respiratory and urinary tracts. It is resistant to ampicillin, macrolides and first generation cephalosporins (Kayla Buchholz *et al.*, 2004).

**APPLICATION TO PROTEOLYTIC ENZYME SERRAPEPTIDASE**

In vivo effects of proteolytic enzyme serratiopeptidase on sputum collected from bronchitis rabbits were examined. Serratiopeptidase (20 mg/kg) (30 mg/kg) significantly reduced the viscosity of sputum (*p*<0.05) after the 1-3-h periods and the 4-6-h periods, respectively, after intra duodenal Administration. 50 mg/kg of serratiopeptidase also significantly decreased not only viscosity (*P*<0.001) but also amount of freeze-dried substance (*p*<0.05) of sputum at the 1-3-h periods. The enzyme serratiopeptidase significantly increased the volume of sputum, probably as the result of liquefaction (Hans Nieper *et al.*, 1999).

Thus, mucolytic expectorant activity of enzyme can be demonstrated first by the reduction in viscosity and next by the increase in volume of sputa. However, the decrease in amount of freeze-dried substance is not always in accord with the reduction in viscosity.

The same enzyme used was also an effective mucolytic in the treatment of various disorders related to viscous sputum or pus, and
their efficacies have been warranted to be more potent and reliable than those of a chymotrypsin and others. Therefore, they have widely been used not only in Japan but also in some other countries. Nevertheless, the pharmacological evidence which substantiates their clinical efficacies, in particular, mucolytic expectorant effect, is insufficient, though they exhibit potent mucolytic activity in vitro experiments. Further pharmacological study, for instance, the acting mechanism of mucolytic expectorant effect of intra duodenally administered enzyme was very effective.

The use of enzyme with fibrinolytic, proteolytic and anti-endemic activities has gained increasing support in recent years for the treatment of inflammatory ear, nose and throat (ENT) conditions.

Included among these enzymes is the Serratia peptidase, a protease obtained from nonpathogenic enterobacteria of the genus Serratia.

This proteolytic enzyme, which is available in tablet form to enable it to be absorbed from the intestinal lumen, has been shown to induce intense fibrinolysis.

Anti inflammatory, and anti-endemic activity in a number of tissues and results suggest that its anti-inflammatory activity may be have particular use for the treatment of localized or 'closed' forms of inflammation, such as those frequently found in ENT pathologies. Another important feature of Serratiopeptidase is its effects on pain, the enzyme acting by inhibiting the release of pain - inducing amines, such as bradykinin, from inflammed tissue (Hans Nieper et al., 1999).
SERRATIOPEPTIDASE CAN HELP

- Fibromyalgia - Arthritis - Chronic Joint Pain
- Chronic Fatigue
- Chronic Pain - Inflammation
- Clogged Arteries - Fibroids Spider Veins - Viral infection
- Circulatory disorders and Systemic yeast Infection
- Autoimmune Diseases
- Post-Operate Scarring
- Fibrocystic Breast Disease
- Bladder Infections

CLINICAL USES OF SERRATIOPEPTIDASE

Serratiopeptidase has many clinical uses including:

- An anti-inflammatory agent (particularly for post traumatic swelling)
- For Fibrocystic breast disease
- For Bronchitis (Serratiopeptidase loosens and expels mucous)
- Serratiopeptidase digests dead tissue, blood clots, cysts, and arterial plaque.

The late German physician Dr. Hans Nieper, 1999, used serratiopeptidase to treat arterial blockage in his coronary patients. Clinical studies show that serratiopeptidase have fibrinolytic, anti-inflammatory and anti-endemic (prevent swelling and fluid retention) activity in a number of tissues, and that its anti-inflammation effects are superior to other proteolytic enzymes.
Besides reducing inflammation, one of serratiopeptidase’s most profound benefits is reduction of pain, due to its ability to block the release of pain-inducing amines from inflamed tissues. Physicians throughout Europe and Asia have recognized the anti-inflammatory and pain-blocking benefits of this naturally occurring substance and are using it in treatment as an alternative to salicylates, and other NSAIDs.

The knowledge of production, purification and characterization of Serratiopeptidase is very important for improvising the activity and the commercial value of the enzyme. The controlled fermentation of *Serratia sp.* secretes serratiopeptidase enzyme in the highly selective medium. The recovery process involves various types of filtration, concentration and steps to make enzyme useful for pharmaceutical applications and finally dried to a fine free flowing powder form.

Serratiopeptidase is an endopeptidase, having molecular weight of about 60 K Dalton. It absorbs strongly at 275-280 nm. Serratiopeptidase is a stronger caseinolytic agent than any other known alkaline or neutral protease.

The medicinal use of Serratiopeptidase is very well known and very well documented. Recent Japanese patents even suggest that oral serratiopeptidase may help treat or prevent such viral diseases as AIDS, Hepatitis B and C. But perhaps its most spectacular application is in reversing cardiovascular disease. In fact, serratiopeptidase appear so effective in unblocking carotid arteries that one researcher—Dr. Hans Nieper, the late, eminent internist from Hannover, Germany—called it a “miracle” enzyme (Hans Nieper *et al.*, 1999).
The proteolytic enzymes in common use today is derived from bacteria (serratiopeptidase grown from *Serratia marcescens* cultures), plants (bromelain from pineapple stem and papain from papaya), and animal sources (trypsin and chymotrypsin from hogs or cattle) (Andrade *et al.*, 2002).

They’re all generally useful, but for many applications serratiopeptidase appears to be the most useful of them all. In one study serratiopeptidase was compared to trypsin, chymotrypsin, and pronase (another microbial peptidase) in a rat model of scalding, which is known to induce abnormal activation of fibrinolysis (Kayla Buchholz *et al.*, 2004).

Serratiopeptidase is a proteolytic enzyme available for clinical use more than a decade. Serratiopeptidase binds to alpha -2-macroglobulin in the blood in the ratio of 1:1, which helps to mask its antigenicity but retains its enzymatic activity and is slowly, transferred to site of inflammation. Serratiopeptidase hydrolyses bradykinin, histamine and serotonin responsible for the oedematic status. Serratiopeptidase reduces swelling, improves microcirculation and expectoration of sputum etc., (Anilkumar and Sadhana Nighojkar, 2005).

Serratiopeptidase when consumed in unprotected form is destroyed by acid in the stomach. However, enteric coat of tablet enable the enzyme to pass through the stomach unchanged and absorb in the intestine.
The enzyme is used in the following clinical conditions.

- In sports injuries, fractures, dislocation and osteoarthritis.
- Serratiopeptidase reduces inflammation and helps in faster healing and repair.
- Serratiopeptidase reduces post operative edema at injection sites.
- Serratiopeptidase reduces internal tissue edema and inflammation caused at post-operative handling. Reduction in edema reduces chances of rupture at tissue as well as risk in case of plastic surgery and graft rejection.
- Serratiopeptidase has mucolytic activity in sinuses, ear cavities and anti-inflammatory activity in upper respiratory tract organs and helps in faster resolution, better antibiotic bioavailability and faster cure rates.
- Serratiopeptidase is used in acute painful inflamed dermatitis.

Serratiopeptidase helps in better control over dental infections and inflammation. The anti-inflammatory activity of serratiopeptidase helps in resolution of post-partum haematomas, breast engorgements and pregnancy related thrombophlebitis.

Serratiopeptidase is an endopeptidase and is a strong caseinolytic agent than any other proteases. Farmer and Daniel, (1972) *Serratia marcescens* from rain water was isolated using Deoxyribonuclease - Toluidine Blue-Cephalothin agar and the pigment production was confirmed by milk agar.
The main aim of this particular study is to optimize the production of serratiopeptidase from *Serratia marcescens* and innovate a new technique that can minimize the cost production of the enzyme industrially. In this regard the society can be immensely benefitted by the therapeutic value of serratiopeptidase which is in great demand.

The enzyme eyes a future therapy for many of deadly and emerging clinical conditions to which very little or no alternatives are available. With this futuristic approach serratiopeptidase characterization and intensive study on it can pioneer future manipulation of the enzyme for betterment of its performance.