



Review

Serratiopeptidase: Insights into the therapeutic applications

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ABSTRACT

Therapeutic applications of enzymes have been widely accepted in clinical practices for decades. Proteolytic enzymes in particular, have been used for the treatment of diseases and disorders. Serratiopeptidase is a proteolytic enzyme having immense applications in therapeutic areas which have been validated by several *in vitro*, *in vivo*, and clinical studies as well as through anecdotal evidences. These applications are attributable to its versatile properties including anti-inflammatory, anti-biofilm, analgesic, anti-edemic, and fibrinolytic effects. The significant impact of serratiopeptidase reported needs to be backed by more scientific data. This review encompasses the details of therapeutic applications of serratiopeptidase based on available *in vitro*, *in vivo*, and clinical studies. We found some strong evidences regarding the efficacy of serratiopeptidase. However data on safety, tolerability, and its mechanism of action need detailing. This review aims to further explore the available literature on serratiopeptidase as well as provide scientific details for existing applications.

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1. Introduction

Enzymes are an essential part of most metabolic processes and are directly or indirectly important for the normal functioning of the human body. They control many physiological functions such as digestion, metabolism, immune function, reproduction, and respiration. Enzymes are obtained from plant, animal, and microbial sources and currently used in clinical practices for the treatment and management of various diseases and disorders. Enzyme-based therapeutics is recently gaining more attention due to its selectivity, efficiency, and safety profile. The therapeutic efficacy of various enzymes including trypsin, chymotrypsin, papain, and bromelain has been proven [1]. Serratiopeptidase (serralysin/ serratia-protease/serrapeptidase) is a widely used proteolytic enzyme in therapeutic applications. It has shown significant anti-inflammatory, anti-edemic, and analgesic effects in various areas including surgery, orthopaedics, otorhinolaryngology, gynaecology, and dentistry [2]. It is well known amongst researchers for its caseinolytic (proteolytic) and fibrinolytic properties.

Serratiopeptidase is a zinc containing metalloprotease of molecular weight 45–60 kDa. The enzyme has an EC number 3.4.24.40 and belongs to the group Serralysin. It is originally

obtained from *Serratia marcescens* isolated from the intestine of the silkworm *Bombyx mori* L. Extensive review on analytical techniques used in the qualitative and quantitative analysis of serratiopeptidase has been published by [3], where the authors pointed out a need for selective and specific techniques for the quantification of serratiopeptidase. A detailed review of existing evidences for serratiopeptidase has been reported by [4]. The major role of this enzyme against inflammation is well mapped in the review written by [1]. Other applications of serratiopeptidase in clinical practices majorly include breast disease, atherosclerosis, Alzheimer's disease, sinusitis, hepatitis, lung disorders, and uterine fibroids [5].

This review is a comprehensive study of the therapeutic potential of serratiopeptidase as well as its regulatory status. We have included *in vitro*, *in vivo*, and clinical study reports as well as some uncompleted studies from different therapeutic areas to our best capacity. This review helps to elucidate the potential of serratiopeptidase as well as identify lacunas in using it to its full extent and hence, identify areas of future research (Fig. 1).

2. Role of enzymes in therapeutics

In 1964, de Duve first suggested use of enzymes as replacement therapy for genetic disorders [6]. With the emergence of novel diseases and failure of conventional treatments in certain conditions, enzyme-based therapeutics is playing an important role in the current century. Target specificity and multiple quick

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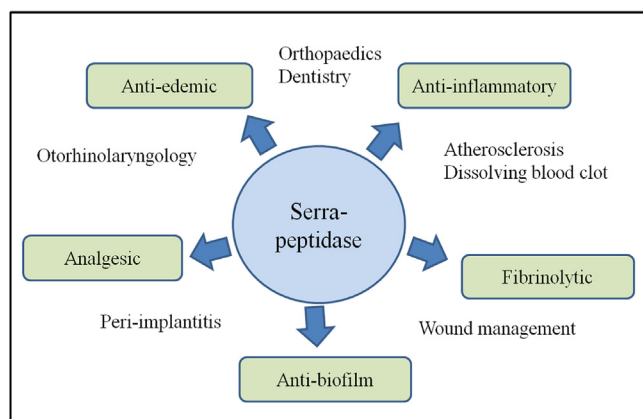


Fig. 1. Properties of serratiopeptidase contributing to its wide applications in therapeutics.

substrate conversion are two main properties which have made enzymes successful and popular over non-enzymatic drugs in therapeutic areas. Enzymes are widely used to treat cancer, cardiovascular diseases, digestive disorders, wound debridement, lysosomal storage disease, inflammatory reaction, genetic disorders, and bleeding disorders [7,8]. Several enzymes have been cited in literature for their therapeutic potential including collagenase, proteases, streptokinase, lysostaphin, laccase, glutaminase, and lipase [9]. Specifically, serine proteases have been used to treat blood vascular disorders; L-asparaginase and L,L-glutaminase have shown efficacy in the treatment of acute lymphoblastic leukemia; and caspases have been used for treating cancer and many classes of viruses [1].

Some obstacles in using enzymes in therapeutic applications include their large size making it difficult for distribution in the body, immunogenicity, short half-life, and impurities. Due to these factors, very few enzymes have been approved by the FDA despite their proven efficacy. Enzymes including alteplase, reteplase, tenecteplase, urokinase, streptokinase, and anistreplase are FDA approved for the treatment of cardiovascular diseases [8]. Few enzymes with notable divergent properties include serratiopeptidase, superoxide dismutase, adenosine deaminase, phenylalanine ammonia lyase, dornase, and rasburicase [8]. Our review highlights the therapeutic molecule serratiopeptidase and its wide clinical applications.

3. Serratiopeptidase

Conventionally, serratiopeptidase is produced from *Serratia marcescens*, a Gram negative opportunistic pathogen in nutrient rich growth medium. The details of production process and media optimization were explained in earlier review [5]. It has been shown to have maximum activity at pH 9.0 and temperature 40 °C and is inactivated at 55 °C in 15 min [2]. It is stable in a wide range of pH (pH 3–10) as revealed in the circular dichroism study, where it showed stable secondary structure [10]. The gene encoding serratiopeptidase is made up of 470 amino acids, devoid of sulfur containing amino acids. The enzyme is produced, purified, characterized and modeled using SWISS-MODEL by [11] where authors have authenticated the structure by assessing the Ramachandran plot using PROCHECK server.

Presently, the demand of serratiopeptidase for the industry and pharmaceuticals is being satisfied by wild or mutant strains of *Serratia marcescens*. However, the pathogenic nature of the organism and hazard associated with the bulk biomass released after fermentation necessitates the research on the development of recombinant molecule. Attempts have been carried out to

express serratiopeptidase genes in *Escherichia coli* using suitable vectors [5]. The failure of many of the attempts attributed to the unregulated intracellular expression of proteases causing cell lysis, growth inhibition, instability of the expression plasmids, lack of protein expression, or deposition of the proteins into non-functional misfolded aggregates [12]. Recently, [12] have demonstrated a production of recombinant serratiopeptidase in *Escherichia coli* successfully. Further, [13] have elucidated the optimized growth media and process conditions for the large scale production of the recombinant serratiopeptidase.

4. Application of serratiopeptidase in therapeutics

Serratiopeptidase has been used by healthcare professionals in Japan and Europe for therapeutic applications for decades. Recently the clinical use of serratiopeptidase alone or in combination with other drugs is increasing worldwide.

4.1. Serratiopeptidase as an anti-inflammatory agent

Inflammation serves as a defense mechanism against injury and infection. The immune system responds rapidly to any foreign substances as well as tissue injury by recruiting immune cells and inflammatory mediators to the target site. Hence, inflammation is considered to be the cleaning process of the body leading to maintenance of homeostasis [14]. Based on the pathologic conditions of the tissue and intensity of the trigger, inflammation can be acute or chronic. Though acute inflammation is a protective measure against injury or infection, failure of its resolution leads to chronic inflammation. Inflammatory disorders such as arthritis, sinusitis, bronchitis, fibrocystic breast disease, and carpal tunnel syndrome etc. are common worldwide. Conventional nonsteroidal anti-inflammatory drugs (NSAIDs) alone or in combination with other drugs are prescribed to combat acute inflammation, whereas steroidal drugs are combined with NSAIDs to treat chronic inflammation [1]. The enormous limitations of these drugs have necessitated research on other possible treatments, including natural molecules. Hence, enzyme-based drugs are now popular in many therapeutic areas including inflammation.

Serine proteases are commonly used in therapeutic areas including inflammation. They were found to have high affinity for cyclooxygenases (COX-I and COX-II), key enzymes in the production of different inflammatory mediators. Serratiopeptidase was first used for its anti-inflammatory effects in Japan in 1957 [1]. Further, many researchers have evaluated the potential of serratiopeptidase against inflammation in different therapeutic areas. It has also been used along with other NSAIDs to achieve a combined effect. Though serratiopeptidase has been proven to be an effective anti-inflammatory molecule in many studies, efforts are needed to optimize its dose based on the application. The concentration of serratiopeptidase in the plasma was found to vary with body mass. Hence, validated cross over studies and optimization are necessary steps to be taken before recommending and prescribing serratiopeptidase [15].

4.1.1. Pre-clinical and clinical studies

[16] compared the anti-inflammatory effect of serratiopeptidase with aspirin and other proteolytic enzymes trypsin and chymotrypsin in albino rats against carrageenan induced paw edema. Serratiopeptidase showed better anti-inflammatory activity alone as well as showed a synergistic effect with aspirin in both acute and subacute models of inflammation in rats. Another small pre-clinical study by [17] on 16 Charles Foster albino rats evaluated the anti-inflammatory effect of serratiopeptidase against diclofenac sodium. Serratiopeptidase at (10–20 mg/kg of body weight) showed comparable results to that of diclofenac

sodium (0.5 mg/kg) in inhibiting acute as well as chronic inflammation in paw edema. The dosage of serratiopeptidase required was higher as compared to diclofenac. This may be due to the low bioavailability of serratiopeptidase, which can be improved using a suitable delivery mode (explained in appropriate section of this review).

Ulcerative colitis is an inflammatory bowel disease caused by overstimulation or inadequate regulation of the mucosal immune system, affecting rectal and colonic mucosa. [18] conducted an impactful study to test the potential of serratiopeptidase on acetic acid-induced ulcerative colitis in mice. Several inflammatory markers including C-reactive protein, myeloperoxidase, glutathione, and nitric oxide were examined, along with histopathological examination. Serratiopeptidase reduced the disease activity index and prevented colonic shortening, spleen enlargement, glutathione depletion, lipid peroxidation, and nitric oxide production as compared to the control group. Further, there was significant reduction in C-reactive protein level in serratiopeptidase treated mice as compared to control. Moreover, myeloperoxidase, an important enzyme marker of inflammation was reduced by serratiopeptidase treatment. These results confirm the anti-inflammatory potential of serratiopeptidase.

There is much clinical data available on the anti-inflammatory effects of serratiopeptidase on pain, swelling, and trismus, details of which are shown in Table 1.

However, some reports on hand showed comparatively lesser effect of serratiopeptidase. In a study by [25] the efficacy and safety of serratiopeptidase and seaprose S in the treatment of venous inflammatory disease were compared. Seaprose S had a higher efficacy as compared to serratiopeptidase (85 % vs 65 %) with no adverse effects. Further, in this study, one out of 20 patients that received serratiopeptidase showed mild gastrointestinal disturbance. [26] found comparatively lesser analgesic and anti-inflammatory effect of serratiopeptidase as compared to Betamethasone and Ibuprofen. Further, a combination of trypsin and

chymotrypsin had a higher efficacy in wound management as compared to serratiopeptidase in a 75 patient study [27]. Contradictory results were observed in two independent studies done on a rat paw edema model. In a study by [28] comparing serratiopeptidase with diclofenac, serratiopeptidase treatment (5.4 mg/kg of body weight) did not result in significant improvement in the inflammation of paw edema. These results are not in agreement with those of [17] where serratiopeptidase (10 mg/kg of body weight) showed promising anti-inflammatory activity. The different dosages used in these two studies make it difficult to draw conclusion.

Overall, serratiopeptidase can be seen as a promising candidate in modern medicine when used either alone or in combination with other agents, particularly in situations where NSAIDs do not show satisfactory results (Tables 2 and 3).

4.2. Serratiopeptidase as an anti-biofilm agent

Bacterial biofilms are multicellular structures of dense and highly hydrated communities of microorganisms embedded within a matrix of self-synthesised polymeric or proteinaceous material [43,44]. They can be attached to biotic or abiotic surfaces. One of the characteristic properties of biofilms is high resistance to the adaptive and innate immune systems as well as tolerance to high concentrations of antibiotics/antimicrobial agents. This leads to persistent infection, making these entities a medical and economic nuisance [31]. They are associated with a variety of infections including urinary tract infections, chronic lung infections, endocarditis, osteomyelitis, chronic otitis media etc. [44]. Biofilms commonly develop on implants and medical devices like catheters, pacemakers, prosthetic joints, tooth surfaces, and various host tissue surfaces leading to chronic infections [44]. They are nearly 100 times more resistant to antimicrobial agents as compared to individual bacterial colonies, thus leading to antibiotic treatment failure.

Table 1

Clinical studies in various therapeutic areas to evaluate efficiency of serratiopeptidase as an anti-inflammatory agent.

Type of Inflammation	Treatment method (Drug(s) taken along with oral administration of serratiopeptidase)	Duration of treatment	Result	Adverse effect reported (Yes/No)	Reference
Knee osteoarthritis	Metformin	5 months	-Reduced pain score -Low level of Tumor necrosis factor alpha (TNF- α), interleukin 1 beta (IL-1 β) and interleukin 8 (IL-8)	No	Ateia et al. [19]
Acne vulgaris	Doxycycline with retin A cream application and panoxyl gel	5 months	Combined therapy showed significantly rapid improvement in acne appearance	-	Mikhael and Mohammed [20]
Postoperative pain, swelling and trismus after surgical removal of mandibular third molars	Methylprednisolone	5 days	Serratiopeptidase showed moderate analgesic activity but effective control of swelling and trismus compared to methylprednisolone	-	Chappi et al. [21]
Postoperative pain, swelling and trismus after surgical extraction of mandibular third molars	-	7 days	Significant reduction in the occurrence of post-surgical swelling and pain, but no effect on postoperative trismus	No	Ai-Khateeb and Nusair [15]
Acute and chronic inflammation of otorhinolaryngology	-	7–8 days	Rapid effect with a significant improvement in symptoms	No	Mazzone et al. [22]
Swelling of the ankle produced by supination trauma	-	5 months	-Swelling decreased by 50 % on the third post-operative day, which was faster than the control group -Serratiopeptidase treated group was rapidly pain free compared to the control group	-	Esch et al. [23]
Postoperative buccal swelling (Caldwell-luc antrotomy)	-	5 days	Serratiopeptidase treated patients showed lesser degree of swelling than placebo-treated patients at every point of observation	No	Tachibana et al. (1984)
Postoperative pain, swelling and trismus after surgical removal of mandibular third molars	-	3 days	Serratiopeptidase showed good effect on trismus but not on swelling and pain	-	Murugesan et al. [24]

Table 2
In vitro studies of a combination of serratiopeptidase and antibiotics for biofilm treatment.

Serratiopeptidase combinations with the antibiotics	Biofilms	Results	Reference
Ofloxacin	<i>Staphylococcus epidermidis</i> <i>Pseudomonas aeruginosa</i>	-Serratiopeptidase enhanced the activity of ofloxacin and inhibited biofilm formation	Selan et al. [29]
Azithromycin	Bacterial biofilms growing on vascular graft surface	- Combination of serratiopeptidase and azithromycin was found to be very effective against <i>Staphylococcus</i> strains by showing less minimum inhibitory concentration (MIC) compared to other antibiotic treatments	Thaller et al. [30]
Ciprofloxacin	<i>Staphylococcus aureus</i>	- Combination of serratiopeptidase (50 µg/mL) and ciprofloxacin at sub-MIC concentration cleared biofilm on catheter.	Gupta and Nagarsenke (2015)
Vancomycin and rifampicin	Methicillin resistant and methicillin susceptible strains of <i>S. aureus</i>	- Effective in dispersing biofilm independent of the strain of organism.	Hogan et al. [31]
Levofloxacin	<i>Staphylococcus aureus</i>	- Eradicated >90 % of the preformed biofilm - Combination showed synergistic activity	Gupta et al. [32]

Table 3
 Mode of delivery of serratiopeptidase for different applications.

Mode of delivery (Entrapment/carrier)	Results	Reference
Serratiopeptidase loaded albumin nanoparticles	Entrapment efficiency and percentage drug release was found to be 85 % and 79.3 % respectively	Kaur and Singh [33]
Encapsulation in liposome	Serratiopeptidase encapsulated in liposome along with antibiotic levofloxacin helps in biofilm irradiation in <i>S. aureus</i> infected rat	Gupta et al. [32]
Clove oil emulsified buccal patch of serratiopeptidase	Significant entrapment efficiency was obtained along with controlled release for 24h	Shende et al. [34]
Poly(D,L-lactic-co-glycolic acid) microspheres of serratiopeptidase and gentamicin entrapped into polyvinyl alcohol-gelatin hydrogel	-The developed delivery system is effective in promoting natural debridement by hydrating necrotic tissue -It provides direct sustained release of antibiotics and serratiopeptidase for better and faster wound healing	Singh and Singh [35]
Serratiopeptidase loaded chitosan nanoparticles	-Sustained release upto 24h -Prolonged anti-inflammatory activity upto 32h	Mali et al. [36]
Microsphere of serratiopeptidase in polymer Eudragit RS100 Liposomal formulations of serratiopeptidase	Prolonged release of serratiopeptidase for sustained therapeutic effect -A maximum entrapment efficiency of 86 % was found -Liposomal formulation of serratiopeptidase improved its permeability as revealed by an <i>in vitro</i> study using PAMPA and caco-2 model	Hire et al. [37] Sandhya et al. [38]
Serratiopeptidase transdermal patch by lipid-based transfersomes	-Entrapment efficiency was 96.76 % - <i>In vitro</i> and <i>in vivo</i> release was controlled and steady	Shende et al. [39]
Serratiopeptidase niosomal gel	-Maximum entrapment efficiency was 54.82 % with a consistent release pattern -Anti-inflammatory activity was comparable to that of diclofenac gel as revealed by <i>in vivo</i> efficacy study	Shinde and Kanojiya [40]
Serratiopeptidase and metronidazole loaded on alginate microspheres by emulsification	-Good loading efficiency -Improved wound healing as observed in <i>in vivo</i> testing in rabbits	Rath et al. [41]
Serratiopeptidase immobilized on amino-functionalized magnetic nanoparticles	-Immobilization increased permeation through the membrane as observed in <i>in vitro</i> studies -Immobilization reduced the dose of serratiopeptidase for anti-inflammatory effect as studied in a rat edema model	Kumar et al. [42]

Various strategies to provide a suitable solution to biofilm associated health problems including inhibition, dispersal, and use of biofilm eradicating agents (antibiotics) have been attempted. Surface proteins and secreted proteins have found to play an important role in biofilm formation, stability, and regulation [44]. Hence, proteases were hypothesized to be a potential treatment of biofilms, which was further supported by scientific studies. Proteases purified from different organisms have tested successfully against biofilm, with metalloproteases in particular, playing an important role [45]. Moreover, commercial proteases have also been successful in the eradication of biofilms [46,47]. Serratiopeptidase, a commercially available bacterial metalloprotease has proven to be effective against a variety of biofilm-associated medical conditions due to the following reasons:

- 1 It can modify the virulent phenotype of bacteria in biofilms [48,49]
- 2 It is effective against mature biofilms [49]

3 It enhances the bactericidal effect of antibiotics against bacterial biofilms [50].

4.2.1. *In vitro* studies

Various *in vitro* studies show a positive impact of serratiopeptidase against biofilms. It affects a discrete number of proteins involved in fundamental mechanisms associated with bacterial virulence, such as adhesion, invasion, and biofilm formation [51]. Serratiopeptidase reduces cell surface proteins Ami4b, autolysin, internalinB, and ActA and hence reduces the ability of *Listeria monocytogenes* to form biofilms and to invade host cells. This leads to the prevention of initial adhesion of *Listeria monocytogenes* to the human gut [52]. [48] tested the role of three serine proteases (proteinase K, trypsin, and chymotrypsin) and two metalloproteases (serratiopeptidase and carboxypeptidase) against biofilm formation and in human cell invasion processes using different strains of *Staphylococcus aureus* and *Staphylococcus epidermidis*.

Among all the proteases tested, only serratiopeptidase was found to inhibit the activity of all the tested strains. It slightly affected the adhesion efficiency (20 %) but drastically reduced the invasion efficiency (200-fold) of *Staphylococcus aureus*. Serratiopeptidase neither affected bacterial viability nor showed any cytotoxic effects on the eukaryotic cell lines, alluding to its safety.

A study of the anti-infective capability of serratiopeptidase against *Staphylococcus aureus* revealed its effects on a discrete number of surface proteins [49]. One such protein is At1 which helps in internalization of staphylococcus in host cells, confirming that serratiopeptidase modulates adhesins and autolysins in *Staphylococcus aureus*. Further, the action of serratiopeptidase is not only restricted to initial bacterial attachment on abiotic surface but is also effective on mature biofilms. The author emphasised that serratiopeptidase hinders the entry of pathogens in human tissue as well as impairs adhesion of pathogens to prostheses, catheters, and medical devices.

[53] developed a mutant form of serratiopeptidase with no proteolytic activity. The developed mutant was found to maintain anti-biofilm property, suggesting that this property is independent of the proteolytic activity of serratiopeptidase. They concluded that serratiopeptidase is a potential antipathogenic agent with or without proteolytic activity and prevents the formation of biofilms on medical devices [53]. Further, authors suggested the need for research to identify the mechanism of action of serratiopeptidase in biofilm regulation that is unrelated to proteolytic activity. Recently, [54] evaluated the anti-biofilm ability of serratiopeptidase against *Staphylococcus aureus* infection on osteoblastic MG-63 cells. The pro-inflammatory chemokine MCP-1 was used as an immunological marker. Serratiopeptidase impaired the invasion capability of *Staphylococcus aureus* in the osteoblastic cells and lowered the secretion of MCP-1. It is worthwhile to note that serratiopeptidase did not affect the viability or proliferation of the osteoblastic cells, indicating its safety in the treatment of bone infection.

Further, combined treatment of serratiopeptidase with antibiotics is a novel approach in the treatment against bacterial biofilm infection. The combination has been shown to have synergistic effects. Serratiopeptidase is thought to work like a biological “nanodrill” and disrupt the bacterial biofilm membrane, thus paving the way for antibiotics to act [32]. Serratiopeptidase was also shown to be more effective as compared to other proteases [29].

4.2.2. Pre-clinical and clinical studies

In an *in vivo* study on 60 Sprague–Dawley rats, serratiopeptidase eradicated periprosthetic infection caused by *Staphylococcus epidermidis* [55]. Histological and microbiological testing revealed that 5.6 % of rats showed infection in the serratiopeptidase + antibiotic treated group vs 37.5 % in the antibiotic alone treated group. The authors concluded that the anti-biofilm property of serratiopeptidase enhances the efficacy of antibiotics. The study reported adverse effects on joint cartilage and synovial tissue attributable to serratiopeptidase. However, the authors believe that serratiopeptidase use is safe as shown in previous studies and commented that a lower dose may not show this adverse effect. Hence, enzyme dosage and application sites should be carefully considered before using an enzyme-antibiotic combination treatment.

Clinical studies of peri-implantitis are important, given the large number of implant surgeries being performed worldwide. Early diagnosis of peri-implantitis is difficult due to initial mild or no symptoms as well as lack of proper diagnostic tests. Antibiotic resistance is another obstacle in its treatment. Colonization of bacteria occurs at the surface of the implant, forming a biofilm which causes severe inflammation of the peri-implant tissue and

damages the implant supporting bone. In a controlled study of 64 adults, [50] evaluated the combined effect of serratiopeptidase with antibiotics in peri-implantitis. The combined treatment significantly improved clinical, microbiological, and inflammatory parameters as compared to the control group. The authors concluded that serratiopeptidase enhanced the efficacy of antibiotics by increasing tissue concentration of the antibiotics [56,57]. A retrospective study in 544 patients was conducted by [58] on partially edentulous patients treated for peri-implantitis by evaluating clinical charts. The study indicated that serratiopeptidase helps in the repair of bone lesions and speeds up the clinical healing process. The authors strongly believe that the co-administration of serratiopeptidase with antibiotics to be a preferred option in peri-implantitis treatment.

4.3. Other applications

The analgesic effect of serratiopeptidase is widely known and has been reported in clinical studies. The ability of serratiopeptidase to hydrolyse bradykinin, histamine, and serotonin contributes to its analgesic activity. Serratiopeptidase was found to relieve pain in patients with root canal treatment [59] and control toothache when emulsified with clove oil [34]. The analgesic activity of serratiopeptidase has also been proven in cases of surgical extraction of mandibular third molars reported by Al-Khateeb and Nusair, 2008.

A clinical trial by [60] on 70 patients with breast engorgement demonstrated that serratiopeptidase treatment resulted in moderate to marked improvement in breast pain, swelling, and induration with no adverse events reported. A prospective trial to explore the application of serratiopeptidase in the treatment of carpal tunnel syndrome showed clinical improvement in 65 % of the patients [61]. Serratiopeptidase has been widely used in Japan as an anti-inflammatory and mucolytic agent. Airway mucus is a viscoelastic gel important for the airway defense system. However, in chronic airway disease, increased secretion of mucus and decreased clearance causes its accumulation. [62] evaluated the effect of serratiopeptidase on viscosity and elasticity of nasal mucus in patients with chronic sinusitis. Serratiopeptidase was found to reduce viscosity but not elasticity of the mucus. Further, study done by [63] demonstrated that the Serrapeptidase could enhance mucus clearance in patients with chronic airway disease. This effect was attributed to the ability of serratiopeptidase to reduce neutrophil count and modify viscoelasticity of the mucus.

Another innovative and interesting application is in the treatment of Alzheimer's disease *via* reduction of amyloidosis. Serratiopeptidase was found to be as effective as nattokinase (an enzyme shown to degrade amyloid fibroid) in relieving Alzheimer's disease pathophysiology in a rat model [64]. Oral administration of an enzyme decreased brain acetylcholinesterase activity, as well as levels of transforming growth factor β , Fas, and interleukin-6, all of which were significantly increased in patients with Alzheimer's disease. These results were confirmed by histological examination of brain tissue. This study demonstrates that serratiopeptidase can down-regulate the amyloidogenic pathway due to its proteolytic, anti-oxidant, and anti-amyloidogenic effects. The study was further supported by a recent reports [65,66] showing dissociation of insulin amyloids by serratiopeptidase both *in vitro* and *in vivo*. Amyloidosis is a result of misfolding of normal cellular protein to protease resistant β -sheets making insoluble aggregates. These aggregates build up in the body and their clearance is highly difficult. The amyloid dissociation ability of serratiopeptidase was better than that of the standard amyloid dissociating agent, nattokinase. This novel approach paves the way to explore the therapeutic potential of serratiopeptidase in different amyloid related disorders.

A combination therapy consisting of enteric coated Vitamin C, extract of *Withaferania somnifera*, and serratiopeptidase has been used to treat thyroid cancer [67]. Complete remission of thyroid tumor was found after 18 months of combination therapy. Serratiopeptidase plays an important role in cleaning the dead cells from the target site leading to an increased rate of killing of tumor cells. This study further highlights the scope for injecting enzyme directly to the target site of a tumor to enhance efficiency.

5. Mechanism of action

Despite its wide therapeutic applications, the mechanism of serratiopeptidase action has not been well elucidated.

Wound healing: Serratiopeptidase helps to thin the fluids in inflamed areas, thus facilitating drainage. This results in reduction of swelling, pain, and enhances tissue repair. Serratiopeptidase also accelerates the healing process due to its unique property of dissolving dead tissue surrounding the injured area without harming living tissue. Further, it hydrolyses bradykinin, histamine, and serotonin which helps to decrease pain and swelling and improve microcirculation, which in turn supports the wound healing process [68].

Anti-inflammatory: Serratiopeptidase acts as an anti-inflammatory agent by regulating inflammatory cytokines and hence the onset of chronic inflammation. It significantly modifies cell adhesion molecules that guide inflammatory cells to the sites of inflammation. It promotes wound healing, repair, and restores the skin temperature at the target inflammation site. It should be noted that serratiopeptidase is more stable and has higher efficacy when used in combination with metal ions like zinc and manganese [1].

Antibiofilm: The anti-biofilm ability of serratiopeptidase is accredited to its capability of modulating the expression of adhesion molecules and reduces cell surface proteins of bacteria [51]. It prevents biofilm formation as well as helps to disperse preformed biofilm. Its anti-biofilm ability helps to enhance the penetration of antibiotics through the resistant biofilm and hence increases susceptibility of biofilms to antibiotics.

Fibrinolytic: Serratiopeptidase is known to dissolve blood clots and atherosclerotic plaques by breaking down fibrin and other dead or damaged tissue [2]. It can also remove deposits of fatty substances, cholesterol, and cellular waste inside the arteries. The fibrinolytic property of serratiopeptidase may also help with the problems of thick blood, risk of stroke, and thrombophlebitis [1].

6. Absorption and safety of serratiopeptidase

Meagre literature is available on the absorption and safety of serratiopeptidase in the human body. It is known that orally taken serratiopeptidase gets absorbed through the intestine and transported into the blood. The intestinal absorption of serratiopeptidase has been tested in rats by evaluating its concentration in plasma, lymph, and inflammatory tissue extract using the sandwich enzyme immunoassay technique. The study showed that the concentration of serratiopeptidase in plasma and lymph is dose dependent. Peak plasma concentration was reached at 0.25–0.5 h after intake and the enzyme was measurable upto 6 h [69]. Further, [70] demonstrated that the concentration of serratiopeptidase was higher in inflammatory tissue than that of in plasma. The authors proposed that serratiopeptidase is absorbed from the intestine and distributed to inflammatory sites via blood or lymph. Serratiopeptidase forms a complex with plasma protease inhibitor alpha-1 macroglobulin in the ratio of 1:1 as observed in a rat study [71]. This binding masks its antigenicity with 20 % retention of its original caseinolytic activity. This complex helps to transport serratiopeptidase via blood to the target sites. The dosage

of serratiopeptidase generally ranges from 10–60 mg per day (2000/mg Unit activity). It is highly recommended to get a prescription for serratiopeptidase from health experts because its dosage requirement varies depending on the application and disease state.

Serratiopeptidase is a natural molecule that is being used for decades, hence commonly considered as safe. The safety of this enzyme in different areas of therapeutics is supported by several studies [15,19,22,48,54,72] in which no side effects or adverse events were reported. However, some studies have reported adverse effects of this molecule, but at a rare frequency. The explanation of the same has been provided above in the respective section of this review. Further, Stevens-Johnson syndrome [73] and buccal space abscess [74] have also been reported as side effects of this molecule. These side effects may be dose dependent or possibly due to a combination effect when used with other drugs. Detailed, scientifically designed controlled clinical studies need to be conducted to further examine the safety profile.

7. Mode of delivery of serratiopeptidase

Common problems associated with drug delivery are poor solubility, toxicity, instability, incompatibility, and poor penetration [75]. Each drug needs a suitable delivery system depending on its characteristics. Serratiopeptidase suffers high risk of enzymatic degradation in the gastrointestinal tract due to its proteinaceous nature. Further, its hydrophilic nature causes low permeability through the intestinal membrane. These factors impose the use of a very high dosage for significant effects. Controlled and sustained release of serratiopeptidase is vital approach to decrease the frequency of dosing and to improve patient compliance. Hence, different delivery modes have been studied including magnetic nanoparticles, microspheres, encapsulation in liposomes, and emulsification. *In vitro* release profiles and *in vivo* efficacy are important parameters need to be studied to develop suitable delivery modes.

New modes of delivery have been evaluated in cases of dentistry and wound healing. Novel effective biocompatible moist system for complete wound management was studied by [35]. Poly (D,L-lactic-co-glycolic acid) microspheres of serratiopeptidase and gentamicin were entrapped into polyvinyl alcohol-gelatin hydrogel. *In vitro* and *in vivo* studies showed the direct sustained release of serratiopeptidase along with antibiotics at the wound site leading to a better and faster healing process. [76] developed a topical formulation of serratiopeptidase in the form of an ointment and a gel and evaluated its anti-inflammatory effects. The authors highlighted that topical application circumvents the drawbacks of oral delivery including anorexia, nausea, and GI disturbance, if any. An optimized formulation was evaluated for *in vitro* release profile and *in vivo* anti-inflammatory action where it showed satisfactory inhibition of ear edema in a rat study. The absence of any allergic reaction in rats supports the safety profile of the serratiopeptidase formulation. Enteric dispersion of serratiopeptidase with the polymer Eudragit has shown promising results for controlled release of the drug [37,77].

8. Regulatory aspects

Though serratiopeptidase is widely used in clinical practice around the globe, its regulatory status varies in different countries. The marketing and use of this molecule in any intended country needs approval by designated regulatory bodies. Background administrative, chemistry, pre-clinical and clinical data are needed for approval where quality, efficacy, and safety are important parameters to be considered. Serratiopeptidase has been approved in Canada for use as an ingredient in dietary supplements to reduce

Table 4
Regulatory status of serratiopeptidase in major global markets.

Country	Regulatory Agency	Approval status	Details	References
USA	U.S. Food & Drug Administration	New Dietary Ingredient Notification (NDIN) Drug Master file (DMF)	NDIN# 6 was filed by Specialty Enzymes & Probiotics DMF# 23,557 filed by Specialty Enzymes & Probiotics and DMF# 27,172 filed by Newgen Biotech	Dietary supplement health and education act (DSHEA) [81] 21 Code of federal regulations (CFR) part- 314
Canada	Health Canada	Natural health product (NHP)	-Enteric coated version is allowed. Can be used for reduction in pain and swelling and to combat throat infections	NHP Ingredients Database: Serrapeptase [78]
Europe	European commission (EC)	Novel Food	Serratiopeptidase is considered as Novel Food. Safety evaluation is needed under 2015/2283 regulation	Regulation (EU) No. 2015/2283
India	Central Drug Standard Control Organization (CDSO)	Approved as active pharmaceutical ingredient (API)	Serratiopeptidase is widely available. It is generally sold in combination with other APIs.	The Drug and Cosmetic Act [80]

pain and swelling [78]. In India, it is approved as a pharmaceutical ingredient for the treatment of acute pain in combination with other drugs [80]. Regulatory authorities provide approval status for specific applications. However, a few regulatory authorities have published approvals for serratiopeptidase to be used as a dietary supplement or as an active pharmaceutical ingredient as listed in Table 4.

Besides, in Japan, proprietor, Takeda has voluntarily withdrawn serratiopeptidase in 2011 then after, Singapore government has taken decision to phase out serratiopeptidase containing preparations as medicinal products due to some controversial results of the trials and lack of substantive scientific evidences [79].

Label/health claim guidelines need to be followed for products containing serratiopeptidase as a dietary ingredient or pharmaceutical ingredient. The big challenge for manufactures and distributors is to understand the array of label claims that are allowed to be used for serratiopeptidase containing products. In the United States, dietary supplements can make structure/function statements [81]. However, regulatory agencies in Europe and Canada have special provisions to allow broader health claims. Those are approved under regulation (EC) no. 1924/2006 in Europe and Food and Drugs act (section 30(j)) in Canada. Manufacturers need detailed case by case evaluation of the approved claims in each country before marketing serratiopeptidase.

9. Conclusion

The proteolytic enzyme serratiopeptidase is being widely used in the treatment of many diseases and disorders for decades across the world. *In vitro* studies, controlled, uncontrolled pre-clinical and clinical studies, and some anecdotal reports highlight its potential in the therapeutics. However, negative and controversial results obtained as well as some reports of mild to moderate side effects cannot be overlooked. Hence, more research is needed to confirm its therapeutic potential, elucidate the mechanism(s) of action as well as safety of different doses and formulations. Well designed *in vitro*, *in vivo*, and clinical studies will help to establish the use of serratiopeptidase in various therapeutic areas.

Author contribution statement

The conception and design of the study: S. J and Abhijit R; acquisition of data, analysis, and interpretation of data: S. J, Ankit R, and N. S; Drafting the article: S. J, N. S, and Ankit R; revising the article critically for important intellectual content: N. S, S. J and V. R; final approval of the version to be submitted: V. R and Abhijit R.

Declaration of Competing Interest

The authors report no declarations of interest.

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