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# A serine proteinase inhibitor isolated from *Tamarindus indica* seeds and its effects on the release of human neutrophil elastase

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**Abstract**

Proteinaceous inhibitors with high inhibitory activities against human neutrophil elastase (HNE) were found in seeds of the Tamarind tree (*Tamarindus indica*). A serine proteinase inhibitor denoted PG50 was purified using ammonium sulphate and acetone precipitation followed by Sephacryl S-300 and Sephadex G-50 gel filtration chromatographies. Inhibitor PG50 showed a $M_r$ of 14.9 K on Sephadex G-50 calibrated column and a $M_r$ of 11.6 kDa on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. PG50 had selective activity while cysteine proteinases (papain and bromelain) and serine proteinases (porcine pancreatic elastase and bovine chymotrypsin) were not inhibited, it was strongly effective against serine proteinases such as bovine trypsin and isolated human neutrophil elastase. The $IC_{50}$ value was determined to be 55.96 $\mu g.mL^{-1}$. PG50 showed neither cytotoxic nor haemolytic activity on human blood cells. After pre-incubation of PG50 with cytochalasin B, the exocytosis of elastase was initiated using PAF and fMLP. PG50 exhibited different inhibition on elastase release by PAF, at 44.6% and on release by fMLP, at 28.4%. These results showed that PG50 preferentially affected elastase release by PAF stimuli and this may indicate selective inhibition on PAF receptors.


*Keywords:* *Tamarindus indica*; Human neutrophil elastase; PAF; fMLP

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## Introduction

Proteinase inhibitors are widely distributed among bacteria, animals and plants. They are present in their reproductive and storage organs and vegetative tissues of most plant families (Ryan, 1990; Shewry and Lucas, 1997). They have regulatory and defensive roles and act as storage proteins (Xavier-Filho, 1993). Among the various groups of proteinase inhibitors, the serine proteinase inhibitors are the most studied and have been isolated from various leguminous seeds (Oliveira et al., 2002; Macedo et al., 2002; Mello et al., 2002; Oliva et al., 2000). Serine proteinase inhibitors are effective against various insect enzymes and therefore have been studied as an alternative approach to pest control (Reckel et al., 1997). Other uses of these inhibitors have been suggested, such as in the prevention of carcinogenesis (Kennedy, 1998). Serine proteinase inhibitors have also been shown to control abnormal exocytosis and secretion of endogenous proteinases which is characteristic of a number of diseases (Kennedy, 1998). The abnormal exocytosis of elastase, a serine proteinase from human neutrophil, is considered to be the primary source of tissue damage associated with inflammatory diseases as pulmonary emphysema (Janoff, 1985; Groutas, 1987; Fujita et al., 1990; Nishi et al., 2003; Ishizawa et al., 2004), adult respiratory distress syndrome (Lee et al., 1981; McGuire et al., 1982; Gossage et al., 1993; Yamazaki et al., 1999; Carney et al., 2001; Nakayama et al., 2002), septic shock (Uchida et al., 1995; Fujie et al., 1999; Dhainaut et al., 2001; Dhainaut, 2002; Devine, 2003), cystic fibrosis (O'Connor et al., 1993; Hansen et al., 1995; Lee and Downey, 2001; McGarvey et al., 2002; Greer et al., 2004), Chronic bronchitis (Fujita et al., 1990; Llewellyn-Jones et al., 1996; Moodley et al., 2000; Lai et al., 2004) and rheumatoid arthritis (Mohr and Wessinghage, 1983; Odagaki et al., 2001; Tur et al., 2004). Compounds which directly inhibit elastase or its release from human neutrophils are of increasing interest in the development of new anti-inflammatory drugs. In the present study, we have isolated and purified proteinaceous inhibitors from seeds of *Tamarindus indica* and examined its effects on human neutrophil elastase (HNE) release by PAF (Platelet activating factor) and fMLP (*N*-formyl-methionyl-leucyl-phenylalanine) stimulus.

## Material and methods

### *Material*

Seeds from *Tamarindus indica* were obtained from IBAMA (Environmental Institute of Brazil) seeds bank in Natal, RN, Brazil. Leukocytes-rich blood from health adults was obtained from HEMONORTE (blood bank), Natal, RN, Brazil. HNE substrate *N*-succinyl-L-alanyl-L-valine-*p*-nitroanilide (SAAVNA) was purchased from Bachem Feinchemikalien AG. Cytochalasin B, PAF (Platelet activating factor), fMLP (*N*-formyl-methionyl-leucyl-phenylalanine), Dimethylformamide from Sigma. Proteinases: Porcine pancreatic elastase, bovine trypsin, bovine chymotrypsin, bromelain and papain were purchased from Sigma. Polidet P-40 was obtained from Polysciences, Inc.

### *Inhibitor isolation and purification*

Finely ground *T. indica* seed meal was extracted with 0.1 M PBS, pH 7.4, for 30 min at room temperature. After centrifugation for 30 min at 12000 g at 4 °C, the supernatant (crude extract) was