ABSTRACT
Bowman Birk inhibitors (BBI) are small protease inhibitors found in the seeds of legumes in particular. Their molecular masses are in the range of 6-9 kDa. They comprise of a binary arrangement of two sub domains with a conserved array of seven disulphide bridges, which play a pivotal role in the stability of the inhibitors. These inhibitors interact simultaneously and independently with two molecules of proteinases. In addition to the protease inhibitor activity, BBI is reported to have anticarcinogenic and radio protective activity and immune stimulating properties. BBIs have also been implicated to play a vital role in plant defense mechanism. Horse gram (Dolichos biflorus) is a pulse crop native to South East Asia and Tropical Africa. Four isoforms of BBIs, from horsegram seeds have been isolated. The inhibitors of horsegram (HGIs) are single polypeptides with a molecular mass of 8.5 kDa. However SDS-PAGE and analytical gel filtration indicate the molecular mass to be 16 kDa, suggesting that they exist as dimers in solution. In contrast, inhibitors of germinated horsegram seeds, HGGIs exist as monomers. The role of active site residue Lys24 and C-terminal end in the dimeric status of the major inhibitor (HGI-III) was previously established by in vitro and homology modeling. To delineate their role in vivo the HGI-III gene was cloned in E. coli and expressed.

HGI-III specific gene was isolated from the genomic DNA of horsegram by PCR based method. The gene was cloned in pRSET C vector such that the extra residues from the vector are avoided. pRSET-rHGI was functionally expressed in E.coli cells and was purified to homogeneity. The characterization of rHGI was carried out and was comparable to the HGI-III already reported. rHGI also existed as a dimer and the kinetic constants of the inhibitor towards trypsin and chymotrypsin were comparable to the HGI-III.
To evaluate the role of active site residue Lys\textsuperscript{24} and the C-terminal end in dimerisation, site directed mutagenesis was performed. Characterization of the inhibitory activities of the mutants revealed that the K24A mutant inhibited elastase instead of trypsin. D75A mutant and Δ76 mutant retained the trypsin inhibitory activity. All the mutants and rHGI exhibited similar chymotrypsin inhibitory activity. The oligomerisation status of the mutants was studied using SDS-PAGE and size exclusion chromatography. The studies pointed that K24A mutant existed as a monomer. The C-terminal mutants, D75A and Δ76 also existed as monomers. Thermal stability studies revealed that the monomers were less stable than the dimers. Thus the cloning and heterologous expression of a functional rHGI provides a platform to unveil the fine specificity of the interactions involved in the dimeric status of HGI-III.

The BBIs have been extensively studied and known to prevent malignant transformation in cancerous cells. The large size of BBIs hinders the bioavailability of orally administered the BBIs. Smaller peptides comprising the inhibitor domain are an attractive alternative. The trypsin inhibitory (TID) domain of horsegram BBI was genetically engineered. The expressed rTID was purified to homogeneity and was evaluated. The apparent molecular mass of the expressed protein was ~4000 Da. The purified inhibitor was stable thermally and also to proteases like pepsin and pancreatin. Kinetic studies indicated that the expressed peptide (rTID) is a non-competitive inhibitor of trypsin and also inhibited tryptase. Preliminary studies revealed that rTID inhibits other trypsin like proteases. This smaller peptide is a better prospect for drug design targeted at tryptase, the enzyme implicated in inflammatory, allergic disorders and multiple sclerosis.