



Elastase inhibition study report

MATERIALS:

1. Elastase Human Neutrophil (Cat No:324681, Sigma-Aldrich, St.Louis, Missouri, United States)
2. 1M-Tris HCl (Cat No: 20-160, Sigma-Aldrich)
3. Methoxysuccinyl-Ala-Ala-pro-val-p-nitroanilide (Cat No: 454454, Sigma-Aldrich)
4. Trypsin Inhibitor, Soyabean (Cat No: 65035, Sigma-Aldrich)
5. Epigallocatechingallate (Cat No:E4143, Sigma)
6. Adjustable multichannel pipettes and a pipettor (Benchtop, USA)
7. 50 ml centrifuge tubes (# 546043 TORSON)
8. 10ml Borosil Glass tubes (TORSON)
9. 10 ml serological pipettes (TORSON)
10. 10 to 1000ul tips (TORSON)

EQUIPMENTS:

1. Pipettes: 2-10 μ l, 10-100 μ l, and 100-1000 μ l.
2. ELX 800 ELISA reader (BioTek, USA)
3. Water bath (Biobee, India)

ASSAY CONTROLS:

- (i) Blank control (Only PBS)
- (ii) Negative control (Only Elastase-17mU/ml)
- (iii) Positive control (Elastase+100ug/ml of EGCG)
- (iv) Solvent control-0.1% DMSO



STEPS FOLLOWED:

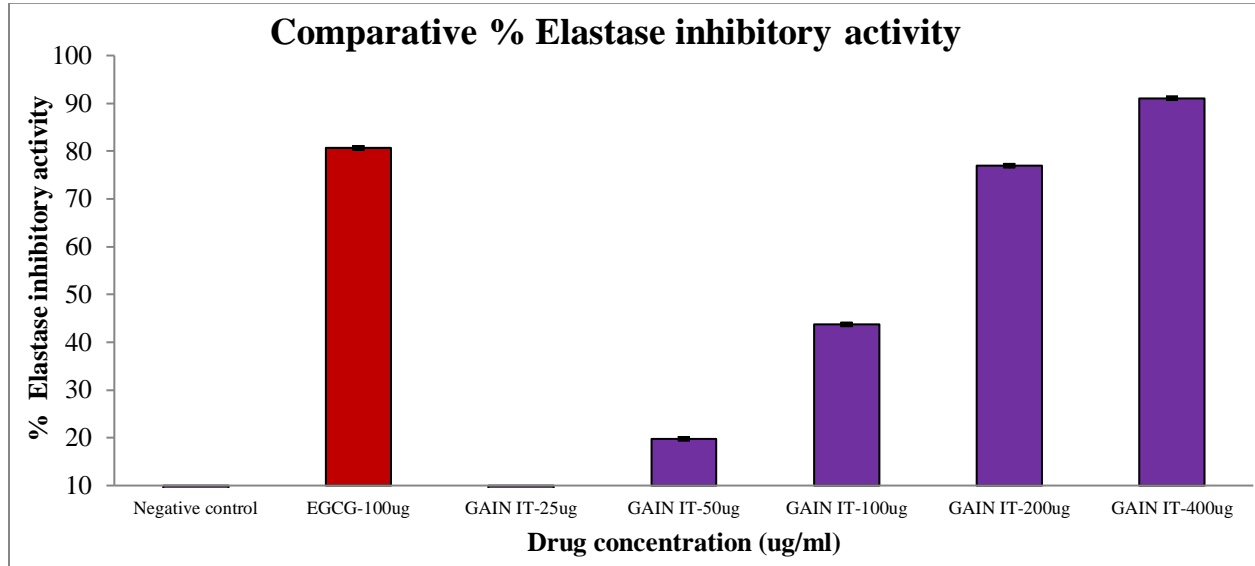
1. Briefly add 1% test compound solution and 17mU/ml of Human Neutrophil Elastase (HNEse) enzyme in Tris-HCl buffer (0.1M with pH 7.5) and incubated the solution for 5min in RT.
2. Afterwards, add equal amount of Methoxysuccinyl-Ala-Ala-pro-val-p-nitroanilide solution to assay reaction mixture and incubated at 37°C for 1hour.
3. Add 500ul of Soyabean Trypsin inhibitor (1mg/ml).
4. Contents in the tubes were then transferred to respective wells of 96well plate.
5. Read the absorbance on a ELISA reader (ELX-800, BioTek) at405nm.
6. **The % Elastaseinhibition activity of the test compound was calculated using the following formula:**

$$\% \text{Elastase inhibition activity} = 100 - \left(\frac{\text{Mean abs Sample}}{\text{Mean abs Control}} \right) \times 100$$

Elastase inhibition studyresults

Culture condition	% Elastaseinhibition activity
Control	0
EGCG-100ug/ml	80.67
GAIN IT-25ug/ml	7.75
GAIN IT-50ug/ml	19.81
GAIN IT-100ug/ml	43.72
GAIN IT-200ug/ml	76.96
GAIN IT-400ug/ml	91.04

Table 1: Table showed the % Elastase inhibition values of the Test Compound, GAIN IT against Elastase after the incubation period of 1hour.Epigallocatechingallate with 100ug/ml was used as a std controls for the study.



Overlaid Bar graph showed the % Elastase inhibitory activity values of GAIN IT.

CONCLUSION OF THE STUDY:

- The Observations in Statistical data of Elastase inhibition study suggesting us that GAIN IT showed significant inhibition of Elastase on dose dependent manner used in the study. Epigallocatechingallate (EGCG) with 100ug/ml was used as a std control for the study which showed 80.67% collagenase inhibition. GAIN IT showed effective Elastase inhibition at 200ug/ml concentration with 76.96±0.01% elastase inhibition.