



Collagenase inhibition study report

MATERIALS:

1. Collagenase (Cat No:C5138, Sigma)
2. TES buffer (Cat No: 14676, SRL)
3. Calcium chloride (Himedia)
4. Epigallocatechingallate (Cat No: E4143, Sigma)
5. EDTA (himedia)
6. Methanol (Cat No: 34860-1L-R, Sigma)
7. FALGPA (Cat No:F5135, Sigma)
8. Citrate buffer (HIMEDIA)
9. Ninhydrin solution (55058,SRL)
10. Isopropanol (SRL)
11. Adjustable multichannel pipettes and a pipettor (Benchtop, USA)
12. 50 ml centrifuge tubes (# 546043 TORSON)
13. 10ml Borosil Glass tubes (TORSON)
14. 10 ml serological pipettes (TORSON)
15. 10 to 1000 ul 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 Collagenase-1mg/ml)
- (iii) Positive control (Epigallocatechingallate with 100ug/ml)
- (iv) Solvent control-0.1% DMSO

STEPS FOLLOWED:

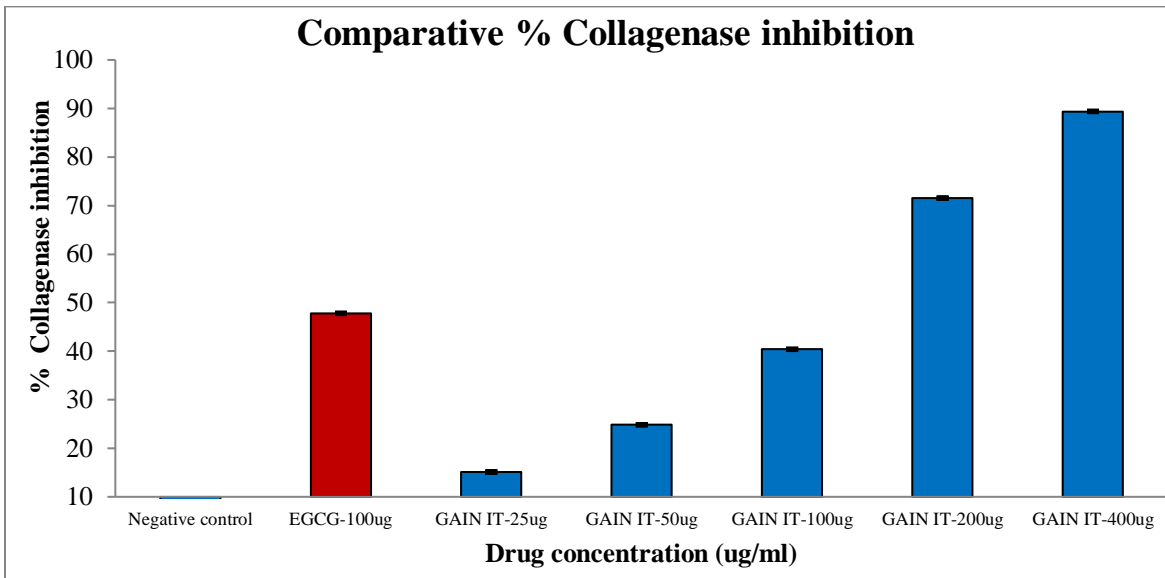
1. The method of Moore and Stein with modifications by Mandl et al was used to determine anti-collagenase activity.
2. Briefly to 2ml test tubes, 25ul of collagenase (1mg/ml), 25ul of TES buffer (50mM) with 0.36mM CaCl₂ (pH-7.4) and 25ul of test sample with various concentrations added.
3. The blank contained 75ul TES buffer while the negative control contained 25ul of collagenase and 50ul of TES buffer.
4. The positive control contained 25ul of collagenase, 25ul of each of TES buffer and 100ug/ml of Epigallocatechingallate (EGCG) while solvent control contained 25ul of collagenase, 25ul of TES buffer and 25ul of 0.1% DMSO in which the samples were dissolved in it.
5. The tubes were incubated in water bath at 37°C for 20 min and afterwards add 100ul of FALGPA to all the tubes and incubated again for 30mins at 37°C.
6. Add 200ul of solution contained citrate buffer (200mM) solution (pH-5) and ninhydrin solution.
7. All the tubes were kept in water bath (100°C) for 5min and left to cool to room temperature before adding 200ul of 50% isopropanol .
8. Contents in the tubes were then transferred to respective wells of 96well plate.
9. Read the absorbance on a ELISA reader (ELX-800, BioTek) at 540nm.
10. **The % collagenase inhibition activity of the test was calculated using the following formula:**

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

Collagenase inhibition study results

Drug conc (ug/ml)	% Collagenase inhibition
Control	0
EGCG-100ug	47.78
GAIN-IT-25ug	15.09
GAIN-IT-50ug	24.85
GAIN-IT-100ug	40.37
GAIN-IT-200ug	71.50
GAIN-IT-400ug	89.38

Table 1: Table showed the % Collagenase inhibition values of the GAIN-IT against Collagenase after the incubation period of 30mins. Epigallocatechingallate with 100ug/ml was used as a std controls for the study.



Overlaid Bar graph showing the % Collagenase inhibition values of GAIN IT

CONCLUSION OF THE STUDY:

- The Observations in Statistical data of Collagenase inhibition study suggesting us that GAIN IT showed significant inhibition of Collagenase on dose dependent manner which concludes the feasibility effective beneficial role of GAIN IT in prohealing at 200ug/ml with $71.50 \pm 0.01\%$.