

Name of Test	Anti-cancer experiment involving Cardiberry™ vs Human submaxillary salivary gland epidermoid carcinoma (HTB-41) cells [Human salivary gland cancer cell]
Aim	To determine the anti-cancer effect of the Cardiberry™ at different dose of exposure vs human submaxillary salivary gland epidermoid carcinoma (HTB-41) cells.
Cell line	HTB-41, human submaxillary salivary gland epidermoid carcinoma.
Cell seeding number	5 x 10 ⁴ cells/well of 6 well plate
Product concentration	20mg/ml and 35mg/ml of Cardiberry™
Duration of product expose to the cells	72 hrs incubation period at 37°C, 5% CO ₂

Test 1: Cardiberry™ vs HTB-41 (Anti-cancer results – 3 days-short term)

Table 1: Cardiberry™ vs HTB-41 cell number and viability

No	Concentration (mg/mL)	No. of viable cell (x104)					Cell Viability				
		R1	R2	R3	Min	SD	R1	R2	R3	Min	SD
1	0	183	177	180	180.00	3.00	100.00	100.00	100.00	100.00	0.00
2	20	116	112	111	113.00	2.65	59.54	65.73	67.15	64.14	4.05
3	35	87	94	91	90.67	3.51	44.72	50.07	52.38	49.06	3.93

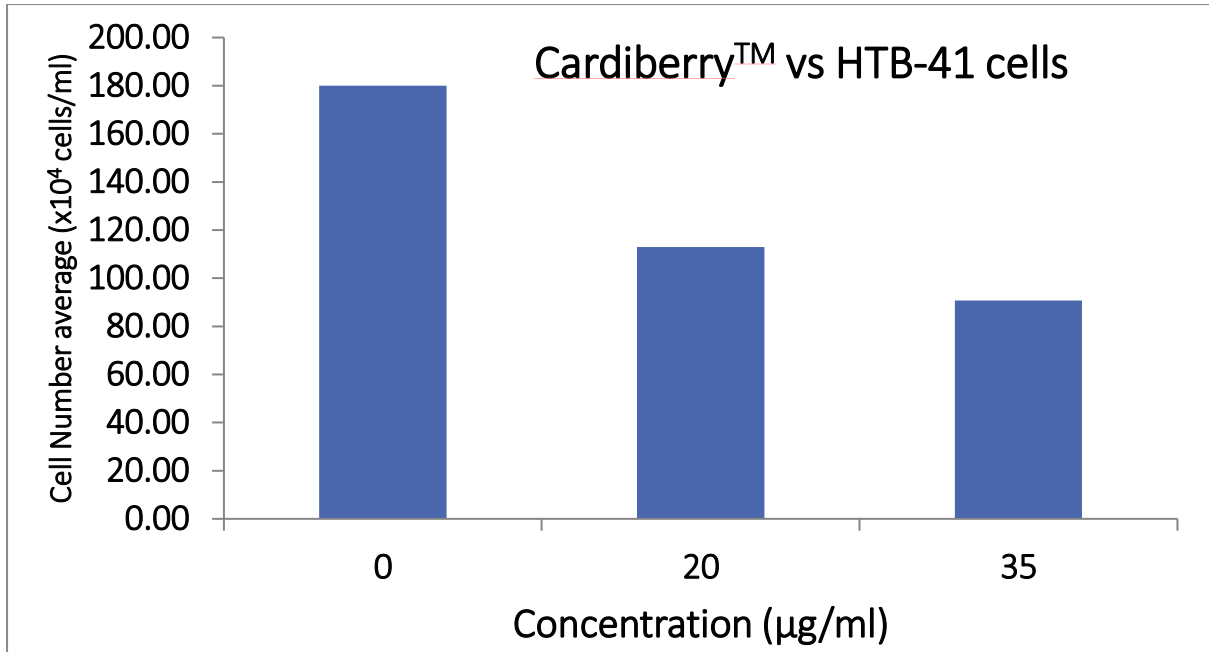


Figure 1: Cell number of HTB 41 cells treated with Cardiberry™

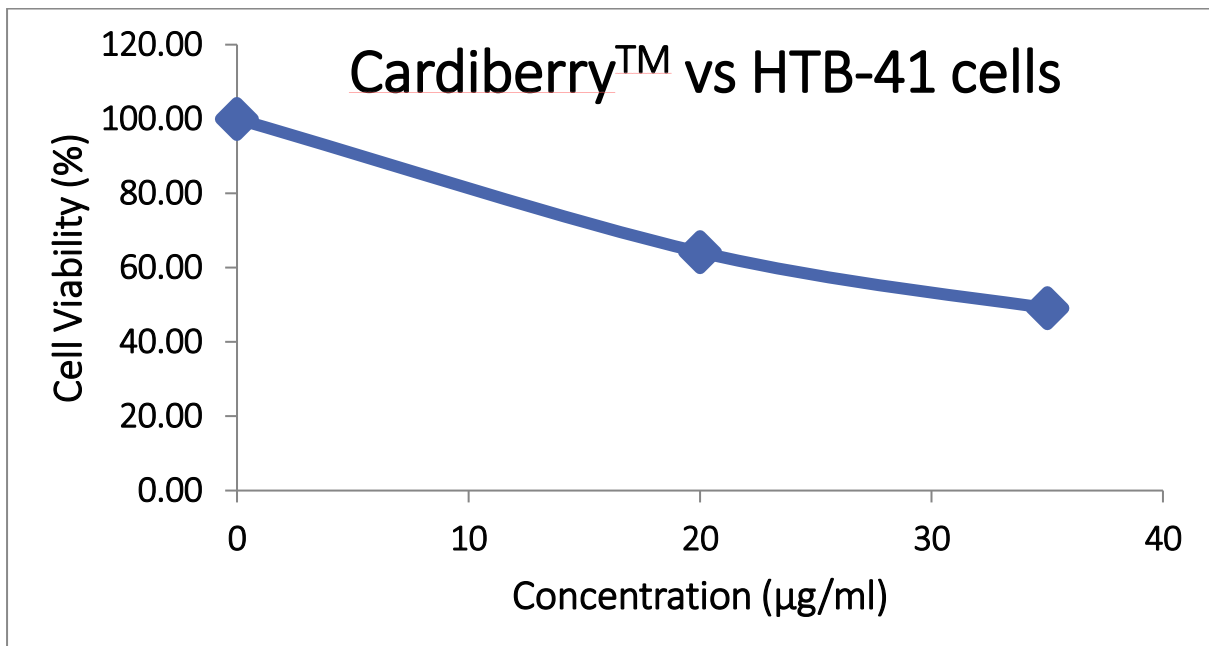


Figure 2: Cell viability of HTB 41 cells treated with Cardiberry™

Anti-cancer experiment was evaluated at 72 hours of treatment using Trypan Blue Exclusion Assay (TBEA). TBEA was used to determine the effect of Cardiberry™ on HTB41 cells. Different concentrations of Cardiberry™ (20mg/ml and 35mg/ml) were tested on HTB 41 cells for 72 hours.

These two doses were selected because:

- 35mg/ml represent the recommended dose (one sachet) for consumption = 7g of Cardiberry™ powder mixed with 200ml of room temperature water.
- 20mg/ml represent the recommended dose (half sachet) for consumption = 4g of Cardiberry™ powder mixed with 200ml of room temperature water.

Cardiberry™ dose of 35mg/ml managed to exhibit highest reduction of number of HTB41 cells from 180.00×10^4 to 90.37×10^4 while Cardiberry™ dose of 20mg/ml managed to exhibit reduction of HTB41 cells from 180.00×10^4 to 113.00×10^4 . Meanwhile cell viability (healthy cancer cells) test indicates Cardiberry™ dose of 35mg/ml managed to reduce cell viability (healthy HTB 41 cancer cells) to 49.06% while Cardiberry™ dose of 20mg/ml managed to reduce cell viability (healthy HTB 41 cancer cells) to 64.14%. This result suggested that Cardiberry™ has a good anti-cancer properties because it managed to reduce the number of healthy cancer cells.

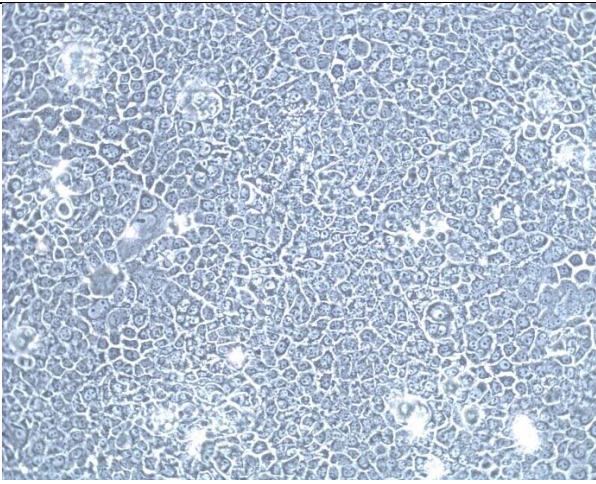
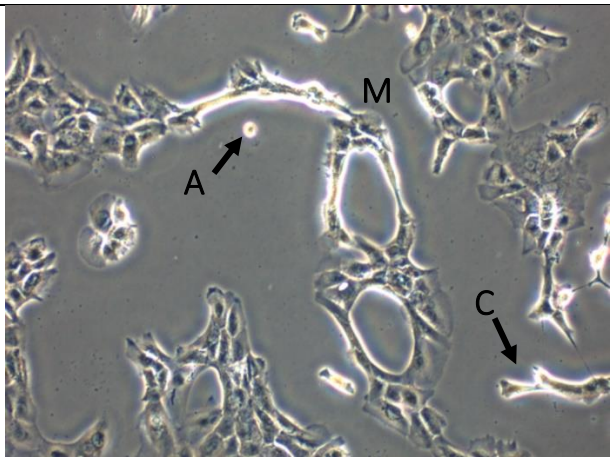
Samples	Magnification (100x)
Untreated	
20mg/ml Cardiberry™	



Figure 3: Cell morphology of HTB 41 cells with and without Cardiberry™ treatment. There are few apoptotic morphological changes seen (100x) such as cell shrinkage (C), membrane blebbing (M) and apoptotic bodies (A).

Test 2: Long term (8 days) – Anti-cancer Effect of Cardiberry™ vs HTB-41

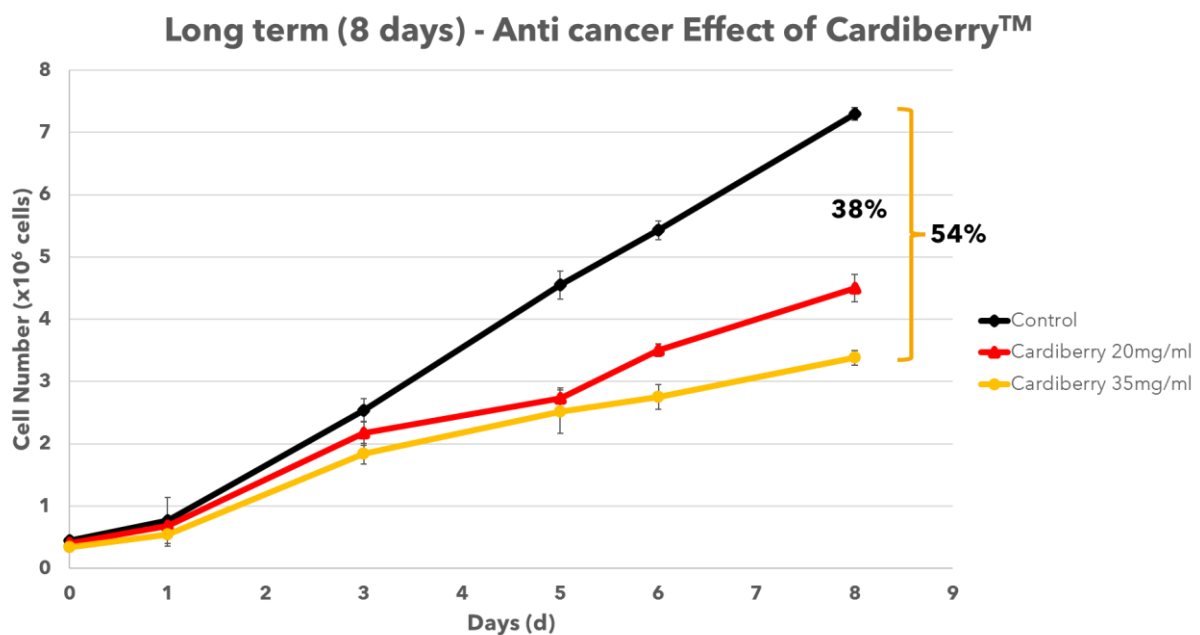



Figure 4: The viable cell numbers determined by using Trypan Blue Exclusion Assay (TBEA) demonstrated that proliferation of HTB 41 cells was significantly suppressed by two doses of Cardiberry™ after 8 days treatment.

The viable (healthy) cell numbers determined using Trypan Blue Exclusion Assay (TBEA) demonstrated that proliferation (growth) of HTB-41 cells was significantly suppressed by Cardiberry™ doses compared to untreated cells after treated for 8

days. Prolonged exposure to the Cardiberry™ at the 20mg/ml and 35mg/ml did not cause total inhibition of the HTB 41 cells, but it clearly suppressed cell growth consistently from day one. The result treatment indicated that Cardiberry™ at 35mg/ml dose had suppressed 54% of cells and Cardiberry™ at 20mg/ml dose with 38% compared to untreated cells after 8 days of incubation. This result suggested that Cardiberry™ has a good anti-proliferative effect because it managed to suppress HTB41 cancer cells growth through 8 days of incubation.

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