APPENDIX

Appendix A – Cell Culture Techniques

I. Cell Line Designation: NG108-15 (ATCC® Catalog No.: HB-12317™)

NG108-15 is a somatic hybrid cell line formed by fusing mouse N18TG2 neuroblastoma cells with rat C6-BU-1 glioma cells in the presence of inactivated Sendai virus. The morphology of the cells is flat, round and loosely adherent on cell culture surface.

Complete Growth Medium:

Dulbecco's Modified Eagle's Medium with 4 mM L-glutamine, 4.5 g/L glucose, 4.0 mg/L pyridoxine-HCl and without sodium pyruvate supplemented with 10% heat-inactivated foetal bovine serum, 100 U/mL penicillin/streptomycin, 100 µM hypoxanthine, 0.4 µM aminopterine and 16 µM thymidine.

Revival Medium:

Complete growth medium described above supplemented with 20% instead of 10% heat-inactivate foetal bovine serum.

Cryoprotectant Medium:

Complete growth medium described above supplemented with 7.5% (v/v) dimethyl sulfoxide (DMSO Hybri-Max®, D2650, Sigma®).

Handling Procedure for Frozen Cells

To ensure the highest level of viability, the vial is thawed and the culture is initiated as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70 ℃. Storage at -70 ℃ will result in loss of viability.

1. The vial is thawed by gentle agitation in a 37 ℃ water bath. To reduce the possibility of contamination, the O-ring and cap are kept out of the water. Thawing should be rapid (approximately 2 min).
2. The vial is removed from the water bath as soon as the contents are thawed, and decontaminated by dipping in or spraying with 70% ethanol.

3. The vial content is transferred to a centrifuge tube containing 9.0 mL revival medium and spun at 1000 rpm for 5 min.
4. The cell pellet is resuspended with revival medium and dispensed into a 25 cm^2 culture flask. To avoid excessive alkalinity of the medium during recovery of the cells, the culture vessel containing the revival medium is placed into the incubator for 15 min to allow the medium to reach its normal pH (7.0 to 7.6) prior to the addition of the vial contents.
5. The culture is incubated at 37 ℃ in a 5% CO_2 incubator.

Subculturing Procedure

The culture is examined with an inverted microscope to check for any evidence of microbial contamination and to determine if the majority of cells are still attached to the bottom of the flask.

1. If pH becomes acidic, cells will detach and grow as a suspension that can be transferred by pipetting.
2. If cells are attached, the culture medium is removed and discarded. 1.0 mL Accutase (PAA Lab GmbH, Austria) and 4.0 mL of phosphate buffer saline solution are added to the flask and cells are observed under inverted microscope until the cell layer is dispersed (5 to 10 min). Cells that are difficult to detach are placed at 37 ℃ to facilitate dispersal.
3. The contents of the flask are removed and transferred aseptically into a centrifuge tube containing 2.0 mL of complete growth medium. The cells are centrifuged at 125 ×g for 5 min.
4. The cell pellet is resuspended in complete growth medium by gently pipetting.
5. Appropriate aliquots of the cell suspension are added to new culture flasks at a subcultivation ratio 1:6 to 1:10.
6. The cultures are incubated at 37 ℃ in a 5% CO_2 incubator.

Medium Renewal

Three times weekly

II. Cell Line Designation: MRC-5 (ATCC® Catalog No.: CCL-171™)

MRC-5 cell line is derived from normal lung tissue of a 14-week-old male fetus. This is a normal diploid human cell line with 46,XY karyotype. The morphology of the cells is fibroblastic and adherent on cell culture surface.

Complete Growth Medium:

Eagle's Minimum Essential Medium with Earle's Salts, non-essential amino acids and 2 mM L-glutamine supplemented with 10% heat-inactivated foetal bovine serum, 1 mM sodium pyruvate, 1.5 g/L sodium bicarbonate, 100 U/mL penicillin/streptomycin and 50 µg/mL of amphotericin B.

Revival Medium:

Complete growth medium described above supplemented with 20% instead of 10% heat-inactivate foetal bovine serum.

Cryoprotectant Medium:

Complete growth medium described above supplemented with 5% (v/v) dimethyl sulfoxide (DMSO Hybri-Max®, D2650, Sigma®).

Handling Procedure for Frozen Cells

To ensure the highest level of viability, the vial is thawed and the culture is initiated as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70 ℃. Storage at -70 ℃ will result in loss of viability.

1. The vial is thawed by gentle agitation in a 37 ℃ water bath. To reduce the possibility of contamination, the O-ring and cap are kept out of the water. Thawing should be rapid (approximately 2 min).
2. The vial is removed from the water bath as soon as the contents are thawed, and decontaminated by dipping in or spraying with 70% ethanol.
3. The vial content is transferred to a centrifuge tube containing 9.0 mL revival medium and spun at 1000 rpm for 5 min.

4. The cell pellet is resuspended with revival medium and dispensed into a 25 cm^2 culture flask. To avoid excessive alkalinity of the medium during recovery of the cells, the culture vessel containing the revival medium is placed into the incubator for 15 min to allow the medium to reach its normal pH (7.0 to 7.6) prior to the addition of the vial contents.
5. The culture is incubated at 37 ℃ in a 5% CO_2 incubator.

Subculturing Procedure

The culture is examined with an inverted microscope to check for any evidence of microbial contamination and to determine if the majority of cells are still attached to the bottom of the flask.

1. The culture medium is removed and discarded.
2. The cell layer is rinsed briefly with 5.0 mL of phosphate buffer saline solution to remove all traces of serum.
3. 1.0 mL Accutase (PAA Lab GmbH, Austria) and 4.0 mL of phosphate buffer saline solution are added to the flask and cells are observed under inverted microscope until the cell layer is dispersed (5 to 10 min). Cells that are difficult to detach are placed at 37 ℃ to facilitate dispersal.
4. The contents of the flask are removed and transferred aseptically into a centrifuge tube containing 2.0 mL of complete growth medium. The cells are centrifuged at 125 ×g for 5 min.
5. The cell pellet is resuspended in complete growth medium by gently pipetting.
6. Appropriate aliquots of the cell suspension are added to new culture flasks at a subcultivation ratio 1:2 to 1:5.
7. The cultures are incubated at 37 ℃ in a 5% CO_2 incubator.

Medium Renewal

Two times weekly

III. Poly-D-lysine Coating in Cell Culture Assays

Reagent

Poly-D-lysine solution (1 mg/mL in H_2O) (Catalogue No.: A-003-E, Millipore Corporation, MA, USA)

Coating of 6-well and 96-well culture plate

The poly-D-lysine solution was diluted immediately before use to the final working concentration 20 μg/mL, using sterile phosphate buffered saline (PBS). Each well was filled with appropriate amount of diluted poly-D-lysine solution (1.5 – 2.0 mL in 6-well plate; 0.1 – 0.2 mL in 96-well plate) to ensure uniform coating of culture surface. After 2 hr of coating at room temperature under sterile conditions, the poly-D-lysine solution was removed. The well surface was thoroughly rinsed with sterile PBS and allowed to dry at least an hour before introducing cells and medium.

Coating of 12 mm glass cover slips

Glass cover slips were sterilized by autoclaving for 15 min at 121 ℃, 15 psi. The poly-D-lysine solution was diluted immediately before use to the final working concentration 50 μg/mL, using sterile phosphate buffered saline (PBS). The cover slips were laid flat in a petri dish and covered with poly-D-lysine solution at 4 ℃ overnight. The cover slips were thoroughly rinsed with sterile distilled H_2O twice and allowed to dry for at least an hour. Coated cover slips were stored at 4 ℃ and exposed to UV light overnight prior assay.

Appendix B – Buffers, Reagents & Solutions

For cell culture

1. Phosphate buffered saline (PBS) (1 × solution)

   1.52 g sodium hydrogen phosphate (Na_2HPO_4)

   0.58 g potassium hydrogen phosphate (KH_2PO_4)

   8.5 g sodium chloride (NaCl)

   Suspended in 1L distilled H_2O and pH adjusted to 7.2 – 7.4

   Solution was filtered with Whatman No.1 filter paper with 11 μm particle retention size and autoclaved for 15 min at 121 ℃, 15 psi.

2. Nerve growth factor (NGF-7S), from mouse submaxillary glands (N0513, Sigma-Aldrich, St. Louis, MO, USA)

   To prepare a stock solution, the content of the vial (0.1 mg) was reconstituted in 10 mL of Dulbecco's Modified Eagle's Medium containing 10% foetal bovine serum. The stock solution was diluted immediately before use to the final working concentration (5 – 200 ng/mL).

   Storage: The stock solution was stored as aliquots in 1 mL for two months at -20 ℃. Prolonged storage or repeated freezing and thawing was avoided to ensure product stability.

3. MTT solution (5 mg/mL)

   5 mg MTT powder (M5655, Sigma-Aldrich, St. Louis, MO, USA)

   1 mL sterile phosphate buffered saline (PBS)

   Solution was filter sterilized using a micropore filter of size 0.2 microns and stored up to 1 month at 4 ℃ in dark.

For NGF Emax® ImmunoAssay System

1. Carbonate coating buffer (pH 9.7)

   0.025M Sodium bicarbonate ($NaHCO_3$)

   0.025M Sodium carbonate (Na_2CO_3)

   pH adjusted to 9.7 using 1N HCl or 1N NaOH.

2. 1N Hydrochloric acid (HCl)

   82.7 mL Concentrated hydrochloric acid

   917.3 mL distilled H_2O

3. 1N Sodium hydroxide (NaOH)

   40.0 g NaOH dissolved in 450 mL distilled H_2O

   Distilled H_2O added to 1L.

4. TBST wash buffer

   20 mM Tris-HCl (pH 7.6)

   150 mM Sodium chloride (NaCl)

   0.05% (v/v) Tween® 20

For immunostaining

1. 4% paraformaldehyde in PBS

   4 g of paraformaldehyde was added to 50 mL of distilled H_2O. The solution was stirred gently on a heating block at 60℃ and 1 mL of 1 M NaOH was added to dissolve the paraformaldehyde. 10mL of 10×PBS was added and the mixture was allowed to cool to room temperature. pH was adjusted to 7.4 and final volume adjusted to 100 mL with H_2O. The solution was filtered through a 0.45 μm membrane filter to remove any particulate matter. Paraformaldehyde solution was prepared fresh prior to use.

2. Washing buffer

   PBS containing 2% sheep serum

3. Blocking buffer

   PBS containing 2% sheep serum and 0.1% Triton X-100

For trypan blue exclusion assay

1. 0.4% trypan blue

   0.4 g of trypan blue powder was added to 100 mL of PBS. The solution was mixed thoroughly and filtered through a 0.45 μm membrane filter to remove any particulate matter. The prepared solution was stored in a srew-capped container at room temperature.

For TUNEL assay

1. 4% paraformaldehyde in 0.1 M NaH_2PO_4 (pH 7.4)

   To prepare 0.1 M NaH_2PO_4, 13.8 g NaH_2PO_4● H_2O was dissolved in 1 L distilled H_2O. 4 g of paraformaldehyde was added to 100 mL of NaH_2PO_4 solution. The mixture was stirred gently on a heating block at 60 ℃ and 1 mL of 1 M NaOH was added to dissolve the paraformaldehyde. The solution was adjusted to pH 7.4 and filtered through a 0.45 μm membrane filter to remove any particulate matter. Paraformaldehyde solution was prepared fresh prior to use.

2. Phosphate buffered saline (PBS) (1 × solution)

1.52 g	sodium hydrogen phosphate (Na_2HPO_4)
0.58 g	potassium hydrogen phosphate (KH_2PO_4)
8.5 g	sodium chloride (NaCl)

   Suspended in 1L distilled H_2O and pH adjusted to 7.2 – 7.4

   Solution was filtered with Whatman No.1 filter paper with 11 μm particle retention size and autoclaved for 15 min at 121 ℃, 15 psi.

3. PBS containing Ca^{2+} and Mg^{2+}

   Solution A

348 mM	sodium hydrogen phosphate (Na_2HPO_4)
70 mM	sodium dihydrogen phosphate (NaH_2PO_4)

   Solution B

18 mM	calcium chloride ($CaCl_2$)
70 mM	potassium chloride (KCl)
18 mM	magnesium chloride ($MgCl_2$)
2740 mM	sodium chloride (NaCl)

   5 mL of solution A was mixed with 5 mL of solution B. The mixture was added with 90 mL of water to prepare PBS with Ca^{2+} and Mg^{2+} (1 × solution). The solution was stored at room temperature.

4. TdT end-labelling cocktail

   TdT buffer, Biotin-dUTP and TdT were mixed at a ratio of 90:5:5. All reagents were provided in the assay kit.

Appendix C – Analytical Techniques

Standard curve in NGF E_{max}® ImmunoAssay System

The NGF standard provided in the system generated a linear standard curve from 3.9 – 250 pg/mL. Absorbance values obtained within the linear range were used to determine the NGF concentration of test samples.

The standard curve demonstrates a direct relationship between the absorbance value and NGF concentration as shown in **Figure C-1**. The direct relationship between the absorbance value and NGF concentration is expressed in the equation y = 0.0021x + 0.0551 (coefficient of determination, R^2 = 0.9983). A standard curve was prepared for each 96-well microplate that is being assayed.

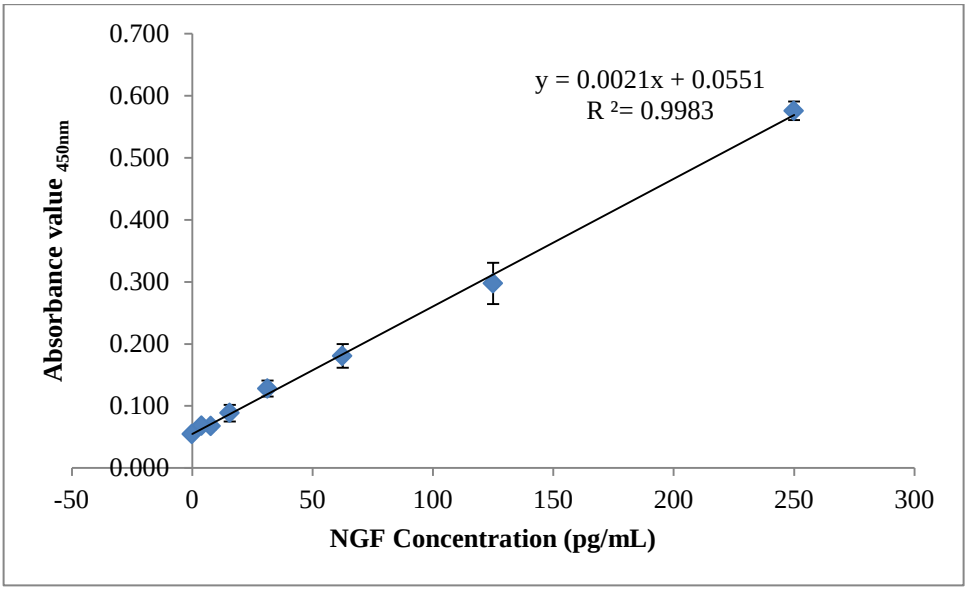

Figure C-1: NGF standard curve for determination of extracellular NGF levels in culture.

Appendix D – Data & Statistical Tables

Table 1: ANOVA: Cytotoxic activity of *H.erinaceus* extract on MRC-5 cell line (between concentrations)

Source	Sum of squares	d.f	Mean square	F-ratio	Significance level
Between groups	92251.00	7	13178.70	153.30	0.0000*
Within groups	5396.29	64	84.317		
Total	97647.30	71			

* $p < 0.05$

Table 2: Multiple range tests: Cytotoxic activity of *H.erinaceus* extract on MRC-5 cell line (between concentrations)

Concentrations	Difference	Limits
0 µg/mL – 0.1 µg/mL	-2.2789	8.6475
0 µg/mL – 1 µg/mL	-1.5033	8.6475
0 µg/mL –10 µg/mL	-11.4322	*8.6475
0 µg/mL – 100 µg/mL	-28.0667	*8.6475
0 µg/mL – 1000 µg/mL	-37.7378	*8.6475
0 µg/mL – 10 000 µg/mL	-0.3233	8.6475
0 µg/mL – 50 000 µg/mL	88.9222	*8.6475
0.1 µg/mL – 1 µg/mL	0.7756	8.6475
0.1 µg/mL –10 µg/mL	-9.1533	*8.6475
0.1 µg/mL – 100 µg/mL	-25.7878	*8.6475
0.1 µg/mL – 1000 µg/mL	-35.4589	*8.6475
0.1 µg/mL – 10 000 µg/mL	1.9556	8.6475
0.1 µg/mL – 50 000 µg/mL	91.2011	*8.6475
1 µg/mL –10 µg/mL	-9.9289	*8.6475
1 µg/mL – 100 µg/mL	-26.5633	*8.6475
1 µg/mL – 1000 µg/mL	-36.2344	*8.6475
1 µg/mL – 10 000 µg/mL	1.1800	8.6475
1 µg/mL – 50 000 µg/mL	90.4256	*8.6475
10 µg/mL – 100 µg/mL	-16.6344	*8.6475
10 µg/mL – 1000 µg/mL	-26.3056	*8.6475
10 µg/mL – 10 000 µg/mL	11.1089	*8.6475
10 µg/mL – 50 000 µg/mL	100.3540	*8.6475
100 µg/mL – 1000 µg/mL	-9.6711	*8.6475
100 µg/mL – 10 000 µg/mL	27.7433	*8.6475
100 µg/mL – 50 000 µg/mL	116.9890	*8.6475
1000 µg/mL – 10 000 µg/mL	37.4144	*8.6475
1000 µg/mL – 50 000 µg/mL	126.6600	*8.6475
10 000 µg/mL – 50 000 µg/mL	89.2456	*8.6475

* denotes a statistically significant difference

Table 3: ANOVA: Cytotoxic activity of *H.erinaceus* extract on NG108-15 cell line (between concentrations)

Source	Sum of squares	d.f	Mean square	F-ratio	Significance level
Between groups	48548.50	7	6935.51	267.56	0.0000*
Within groups	1658.98	64	25.9216		
Total	50207.50	71			

* $p < 0.05$

Table 4: Multiple range tests: Cytotoxic activity of *H.erinaceus* extract on NG108-15 cell line (between concentrations)

Concentrations	Difference	Limits
0 µg/mL – 0.1 µg/mL	3.6956	4.7947
0 µg/mL – 1 µg/mL	0.4400	4.7947
0 µg/mL –10 µg/mL	3.6533	4.7947
0 µg/mL – 100 µg/mL	-1.5022	4.7947
0 µg/mL – 1000 µg/mL	-2.3867	4.7947
0 µg/mL – 10 000 µg/mL	53.2800	*4.7947
0 µg/mL – 50 000 µg/mL	66.6000	*4.7947
0.1 µg/mL – 1 µg/mL	-3.2556	4.7947
0.1 µg/mL –10 µg/mL	-0.0422	4.7947
0.1 µg/mL – 100 µg/mL	-5.1978	*4.7947
0.1 µg/mL – 1000 µg/mL	-6.0822	*4.7947
0.1 µg/mL – 10 000 µg/mL	49.5844	*4.7947
0.1 µg/mL – 50 000 µg/mL	62.9044	*4.7947
1 µg/mL –10 µg/mL	3.2133	4.7947
1 µg/mL – 100 µg/mL	-1.9422	4.7947
1 µg/mL – 1000 µg/mL	-2.8267	4.7947
1 µg/mL – 10 000 µg/mL	52.8400	*4.7947
1 µg/mL – 50 000 µg/mL	66.1600	*4.7947
10 µg/mL – 100 µg/mL	-5.1556	*4.7947
10 µg/mL – 1000 µg/mL	-6.0400	*4.7947
10 µg/mL – 10 000 µg/mL	49.6267	*4.7947
10 µg/mL – 50 000 µg/mL	62.9467	*4.7947
100 µg/mL – 1000 µg/mL	-0.8844	4.7947
100 µg/mL – 10 000 µg/mL	54.7822	*4.7947
100 µg/mL – 50 000 µg/mL	68.1022	*4.7947
1000 µg/mL – 10 000 µg/mL	55.6667	*4.7947
1000 µg/mL – 50 000 µg/mL	68.9867	*4.7947
10 000 µg/mL – 50 000 µg/mL	13.32	*4.7947

* denotes a statistically significant difference

Table 5: ANOVA: Effect of NGF on neurite outgrowth stimulation activity (between concentrations)

Source	Sum of squares	d.f	Mean square	F-ratio	Significance level
Between groups	367.506	5	73.5011	4.69	0.0028*
Within groups	470.293	30	15.6764		
Total	837.799	35			

* $p < 0.05$

Table 6: Multiple range tests: Effect of NGF on neurite outgrowth stimulation activity (between concentrations)

Concentrations	Difference	Limits
0 ng/mL – 5 ng/mL	-4.7167	*4.6685
0 ng/mL – 10 ng/mL	-8.2333	*4.6685
0 ng/mL – 20 ng/mL	-10.1833	*4.6685
0 ng/mL – 50 ng/mL	-6.7167	*4.6685
0 ng/mL – 100 ng/mL	-5.1167	*4.6685
5 ng/mL – 10 ng/mL	-3.5167	4.6685
5 ng/mL – 20 ng/mL	-5.4667	*4.6685

Concentrations	Difference	Limits
5 ng/mL – 50 ng/mL	-2.0000	4.6685
5 ng/mL – 100 ng/mL	-0.4000	4.6685
10 ng/mL – 20 ng/mL	-1.9500	4.6685
10 ng/mL – 50 ng/mL	1.5167	4.6685
10 ng/mL – 100 ng/mL	3.1167	4.6685
20 ng/mL – 50 ng/mL	3.4667	4.6685
20 ng/mL – 100 ng/mL	5.0667	*4.6685
50 ng/mL – 100 ng/mL	1.6000	4.6685

* denotes a statistically significant difference

Table 7: ANOVA: Effect of *H.erinaceus* extract on neurite outgrowth stimulation activity (between concentrations)

Source	Sum of squares	d.f	Mean square	F-ratio	Significance level
Between groups	818.375	5	163.675	14.43	0.0000*
Within groups	340.168	30	11.3389		
Total	1158.54	35			

* $p < 0.05$

Table 8: Multiple range tests: Effect of *H.erinaceus* extract on neurite outgrowth stimulation activity (between concentrations)

Concentrations	Difference	Limits
0 µg/mL – 1 µg/mL	-7.6500	*3.9705
0 µg/mL – 10 µg/mL	-13.8333	*3.9705
0 µg/mL – 50 µg/mL	-14.3667	*3.9705
0 µg/mL – 100 µg/mL	-10.1000	*3.9705
0 µg/mL – 500 µg/mL	-9.8667	*3.9705
1 µg/mL – 10 µg/mL	-6.1833	*3.9705
1 µg/mL – 50 µg/mL	-6.7167	*3.9705
1 µg/mL – 100 µg/mL	-2.4500	3.9705
1 µg/mL – 500 µg/mL	-2.2167	3.9705
10 µg/mL – 50 µg/mL	-0.5333	3.9705
10 µg/mL – 100 µg/mL	3.7333	3.9705
10 µg/mL – 500 µg/mL	3.9667	3.9705
50 µg/mL – 100 µg/mL	4.2667	*3.9705
50 µg/mL – 500 µg/mL	4.5000	*3.9705
100 µg/mL – 500 µg/mL	0.2333	3.9705

* denotes a statistically significant difference

Table 9: ANOVA: Effect of *H.erinaceus* extract combined with 5 ng/mL NGF on neurite outgrowth stimulation activity (between concentrations)

Source	Sum of squares	d.f	Mean square	F-ratio	Significance level
Between groups	262.598	5	52.5196	6.36	0.0004*
Within groups	247.845	30	8.2615		
Total	510.443	35			

* $p < 0.05$

Table 10: Multiple range tests: Effect of *H.erinaceus* extract combined with 5 ng/mL NGF on neurite outgrowth stimulation activity (between concentrations)

Concentrations	Difference	Limits
Control – 0 µg/mL	-5.9000	*3.3891
Control – 1 µg/mL	-7.1833	*3.3891
Control – 10 µg/mL	-7.1500	*3.3891
Control – 50 µg/mL	-8.3167	*3.3891
Control – 100 µg/mL	-6.0333	*3.3891
0 µg/mL – 1 µg/mL	-1.2833	3.3891
0 µg/mL – 10 µg/mL	-1.2500	3.3891
0 µg/mL – 50 µg/mL	-2.4167	3.3891
0 µg/mL – 100 µg/mL	-0.1333	3.3891
1 µg/mL – 10 µg/mL	0.0333	3.3891
1 µg/mL – 50 µg/mL	-1.1333	3.3891
1 µg/mL – 100 µg/mL	1.1500	3.3891
10 µg/mL – 50 µg/mL	-1.1667	3.3891
10 µg/mL – 100 µg/mL	1.1167	3.3891
50 µg/mL – 100 µg/mL	2.2833	3.3891

* denotes a statistically significant difference

Table 11: ANOVA: Effect of *H.erinaceus* extract combined with 10 ng/mL NGF on neurite outgrowth stimulation activity (between concentrations)

Source	Sum of squares	d.f	Mean square	F-ratio	Significance level
Between groups	1080.64	5	216.128	30.96	0.0000*
Within groups	209.435	30	6.98117		
Total	1290.08	35			

* $p < 0.05$

Table 12: Multiple range tests: Effect of *H.erinaceus* extract combined with 10 ng/mL NGF on neurite outgrowth stimulation activity (between concentrations)

Concentrations	Difference	Limits
Control – 0 µg/mL	-8.8833	*3.1154
Control – 1 µg/mL	-16.1333	*3.1154
Control – 10 µg/mL	-15.6667	*3.1154
Control – 50 µg/mL	-12.3667	*3.1154
Control – 100 µg/mL	-8.0667	*3.1154
0 µg/mL – 1 µg/mL	-7.2500	*3.1154
0 µg/mL – 10 µg/mL	-6.7833	*3.1154
0 µg/mL – 50 µg/mL	-3.4833	*3.1154
0 µg/mL – 100 µg/mL	0.8167	3.1154
1 µg/mL – 10 µg/mL	0.4667	3.1154
1 µg/mL – 50 µg/mL	3.7667	*3.1154
1 µg/mL – 100 µg/mL	8.0667	*3.1154
10 µg/mL – 50 µg/mL	3.3000	*3.1154
10 µg/mL – 100 µg/mL	7.6000	*3.1154
50 µg/mL – 100 µg/mL	4.3000	*3.1154

* denotes a statistically significant difference

Table 13: ANOVA: Effect of *H.erinaceus* extract combined with 20 ng/mL NGF on neurite outgrowth stimulation activity (between concentrations)

Source	Sum of squares	d.f	Mean square	F-ratio	Significance level
Between groups	474.171	5	94.8343	4.19	0.0053*
Within groups	679.418	30	22.6473		
Total	1153.59	35			

* $p < 0.05$

Table 14: Multiple range tests: Effect of *H.erinaceus* extract combined with 20 ng/mL NGF on neurite outgrowth stimulation activity (between concentrations)

Concentrations	Difference	Limits
Control – 0 µg/mL	-9.6667	*5.6113
Control – 1 µg/mL	-10.2167	*5.6113
Control – 10 µg/mL	-10.6833	*5.6113
Control – 50 µg/mL	-8.4667	*5.6113
Control – 100 µg/mL	-7.4500	*5.6113
0 µg/mL – 1 µg/mL	-0.5500	5.6113
0 µg/mL – 10 µg/mL	-1.0167	5.6113
0 µg/mL – 50 µg/mL	1.2000	5.6113
0 µg/mL – 100 µg/mL	2.2167	5.6113
1 µg/mL – 10 µg/mL	-0.4667	5.6113
1 µg/mL – 50 µg/mL	1.7500	5.6113
1 µg/mL – 100 µg/mL	2.7667	5.6113
10 µg/mL – 50 µg/mL	2.2167	5.6113
10 µg/mL – 100 µg/mL	3.2333	5.6113
50 µg/mL – 100 µg/mL	1.0167	5.6113

* denotes a statistically significant difference

Table 15: ANOVA: NGF levels in NG108-15 cells treated with *H.erinaceus* extract (between concentrations)

Source	Sum of squares	d.f	Mean square	F-ratio	Significance level
Between groups	1609.79	5	321.959	249.54	0.0000*
Within groups	7.74125	6	1.2902		
Total	1617.54	11			

* $p < 0.05$

Table 16: Multiple range tests: NGF levels in NG108-15 cells treated with *H.erinaceus* extract (between concentrations)

Concentrations	Difference	Limits
0 µg/mL – 1 µg/mL	-12.5100	*2.7794
0 µg/mL – 10 µg/mL	-11.9600	*2.7794
0 µg/mL – 50 µg/mL	-21.0550	*2.7794
0 µg/mL – 100 µg/mL	-5.3300	*2.7794
0 µg/mL – 500 µg/mL	15.5400	*2.7794
1 µg/mL – 10 µg/mL	0.5500	2.7794
1 µg/mL – 50 µg/mL	-8.5450	*2.7794
1 µg/mL – 100 µg/mL	7.1800	*2.7794
1 µg/mL – 500 µg/mL	28.0500	*2.7794
10 µg/mL – 50 µg/mL	-9.0950	*2.7794
10 µg/mL – 100 µg/mL	6.6300	*2.7794

Concentrations	Difference	Limits
10 µg/mL – 500 µg/mL	27.5000	*2.7794
50 µg/mL – 100 µg/mL	15.7250	*2.7794
50 µg/mL – 500 µg/mL	36.5950	*2.7794
100 µg/mL – 500 µg/mL	20.8700	*2.7794

* denotes a statistically significant difference

Table 17: ANOVA: NGF levels in NG108-15 cells treated with *H.erinaceus* extract combined with 10 ng/mL NGF (between concentrations)

Source	Sum of squares	d.f	Mean square	F-ratio	Significance level
Between groups	25755700	5	5151140	237.55	0.0000*
Within groups	260211.0	12	21684.3		
Total	26015900	17			

* $p < 0.05$

Table 18: Multiple range tests: NGF levels in NG108-15 cells treated with *H.erinaceus* extract combined with 10 ng/mL NGF (between concentrations)

Concentrations	Difference	Limits
Control – 0 µg/mL	-644.46	*261.9680
Control – 1 µg/mL	-2506.34	*261.9680
Control – 10 µg/mL	-2485.48	*261.9680
Control – 50 µg/mL	-3158.13	*261.9680
Control – 100 µg/mL	-2961.43	*261.9680
0 µg/mL – 1 µg/mL	-1861.88	*261.9680
0 µg/mL – 10 µg/mL	-1841.02	*261.9680
0 µg/mL – 50 µg/mL	-2513.67	*261.9680
0 µg/mL – 100 µg/mL	-2316.97	*261.9680
1 µg/mL – 10 µg/mL	20.8567	261.9680
1 µg/mL – 50 µg/mL	-651.79	*261.9680
1 µg/mL – 100 µg/mL	-455.09	*261.9680
10 µg/mL – 50 µg/mL	-672.65	*261.9680
10 µg/mL – 100 µg/mL	-475.95	*261.9680
50 µg/mL – 100 µg/mL	196.70	261.9680

* denotes a statistically significant difference

Table 19: ANOVA: Effect of H_2O_2 on cell viability after 2 hr incubation (between concentrations)

Source	Sum of squares	d.f	Mean square	F-ratio	Significance level
Between groups	39269.6	8	4908.71	122.15	0.0000*
Within groups	1085.04	27	40.1867		
Total	40354.7	35			

* $p < 0.05$

Table 20: Multiple range tests: Effect of H_2O_2 on cell viability after 2 hr incubation (between concentrations)

Concentrations	Difference	Limits
Control – 0.5 µM	5.9575	9.19748
Control – 1 µM	-2.3025	9.19748
Control – 5 µM	5.1725	9.19748
Control – 10 µM	-0.7350	9.19748
Control – 50 µM	15.7350	*9.19748

Concentrations	Difference	Limits
Control – 100 µM	45.1100	*9.19748
Control – 250 µM	80.9700	*9.19748
Control – 500 µM	83.4275	*9.19748
0.5 µM – 1 µM	-8.2600	9.19748
0.5 µM – 5 µM	-0.7850	9.19748
0.5 µM – 10 µM	-6.6925	9.19748
0.5 µM – 50 µM	9.7775	*9.19748
0.5 µM – 100 µM	39.1525	*9.19748
0.5 µM – 250 µM	75.0125	*9.19748
0.5 µM – 500 µM	77.4700	*9.19748
1 µM – 5 µM	7.4750	9.19748
1 µM – 10 µM	1.5675	9.19748
1 µM – 50 µM	18.0375	*9.19748
1 µM – 100 µM	47.4125	*9.19748
1 µM – 250 µM	83.2725	*9.19748
1 µM – 500 µM	85.7300	*9.19748
5 µM – 10 µM	-5.9075	9.19748
5 µM – 50 µM	10.5625	*9.19748
5 µM – 100 µM	39.9375	*9.19748
5 µM – 250 µM	75.7975	*9.19748
5 µM – 500 µM	78.2550	*9.19748
10 µM – 50 µM	16.4700	*9.19748
10 µM – 100 µM	45.8450	*9.19748
10 µM – 250 µM	81.7050	*9.19748
10 µM – 500 µM	84.1625	*9.19748
500 µM – 100 µM	29.3750	*9.19748
50 µM – 250 µM	65.2350	*9.19748
50 µM – 500 µM	67.6925	*9.19748
100 µM – 250 µM	35.8600	*9.19748
100 µM – 500 µM	38.3175	*9.19748
250 µM – 500 µM	2.4575	9.19748

* denotes a statistically significant difference

Table 21: ANOVA: Effect of H_2O_2 on cell viability after 4 hr incubation (between concentrations)

Source	Sum of squares	d.f	Mean square	F-ratio	Significance level
Between groups	56247.3	8	7030.91	309.75	0.0000*
Within groups	612.868	27	22.6988		
Total	56860.1	35			

* $p < 0.05$

Table 22: Multiple range tests: Effect of H_2O_2 on cell viability after 4 hr incubation (between concentrations)

Concentrations	Difference	Limits
Control – 0.5 µM	-3.8225	6.9124
Control – 1 µM	0.5525	6.9124
Control – 5 µM	2.2075	6.9124
Control – 10 µM	-12.565	*6.9124
Control – 50 µM	21.9575	*6.9124
Control – 100 µM	39.4950	*6.9124

Concentrations	Difference	Limits
Control – 250 µM	94.3175	*6.9124
Control – 500 µM	96.2275	*6.9124
0.5 µM – 1 µM	4.3750	6.9124
0.5 µM – 5 µM	6.0300	6.9124
0.5 µM – 10 µM	-8.7425	*6.9124
0.5 µM – 50 µM	25.7800	*6.9124
0.5 µM – 100 µM	43.3175	*6.9124
0.5 µM – 250 µM	98.1400	*6.9124
0.5 µM – 500 µM	100.0500	*6.9124
1 µM – 5 µM	1.6550	6.9124
1 µM – 10 µM	-13.1175	*6.9124
1 µM – 50 µM	21.4050	*6.9124
1 µM – 100 µM	38.9425	*6.9124
1 µM – 250 µM	93.7650	*6.9124
1 µM – 500 µM	95.6750	*6.9124
5 µM – 10 µM	-14.7725	*6.9124
5 µM – 50 µM	19.7500	*6.9124
5 µM – 100 µM	37.2875	*6.9124
5 µM – 250 µM	92.1100	*6.9124
5 µM – 500 µM	94.0200	*6.9124
10 µM – 50 µM	34.5225	*6.9124
10 µM – 100 µM	52.0600	*6.9124
10 µM – 250 µM	106.8820	*6.9124
10 µM – 500 µM	108.7930	*6.9124
500 µM – 100 µM	17.5375	*6.9124
50 µM – 250 µM	72.3600	*6.9124
50 µM – 500 µM	74.2700	*6.9124
100 µM – 250 µM	54.8225	*6.9124
100 µM – 500 µM	56.7325	*6.9124
250 µM – 500 µM	1.9100	6.9124

* denotes a statistically significant difference

Table 23: ANOVA: Effect of H_2O_2 on cell viability after 6 hr incubation (between concentrations)

Source	Sum of squares	d.f	Mean square	F-ratio	Significance level
Between groups	46225.5	8	5778.19	109.60	0.0000*
Within groups	1423.52	27	52.7229		
Total	47649.1	35			

* $p < 0.05$

Table 24: Multiple range tests: Effect of H_2O_2 on cell viability after 6 hr incubation (between concentrations)

Concentrations	Difference	Limits
Control – 0.5 µM	8.5350	10.5348
Control – 1 µM	10.8700	*10.5348
Control – 5 µM	5.6725	10.5348
Control – 10 µM	6.0450	10.5348
Control – 50 µM	38.6025	*10.5348
Control – 100 µM	67.4450	*10.5348
Control – 250 µM	91.2500	*10.5348

Concentrations	Difference	Limits
Control – 500 µM	90.8800	*10.5348
0.5 µM – 1 µM	2.3350	10.5348
0.5 µM – 5 µM	-2.8625	10.5348
0.5 µM – 10 µM	-2.4900	10.5348
0.5 µM – 50 µM	30.0675	*10.5348
0.5 µM – 100 µM	58.9100	*10.5348
0.5 µM – 250 µM	82.7150	*10.5348
0.5 µM – 500 µM	82.3450	*10.5348
1 µM – 5 µM	-5.1975	10.5348
1 µM – 10 µM	-4.8250	10.5348
1 µM – 50 µM	27.7325	*10.5348
1 µM – 100 µM	56.5750	*10.5348
1 µM – 250 µM	80.3800	*10.5348
1 µM – 500 µM	80.0100	*10.5348
5 µM – 10 µM	0.3725	10.5348
5 µM – 50 µM	32.9300	*10.5348
5 µM – 100 µM	61.7725	*10.5348
5 µM – 250 µM	85.5775	*10.5348
5 µM – 500 µM	85.2075	*10.5348
10 µM – 50 µM	32.5575	*10.5348
10 µM – 100 µM	61.4000	*10.5348
10 µM – 250 µM	85.2050	*10.5348
10 µM – 500 µM	84.8350	*10.5348
500 µM – 100 µM	28.8425	*10.5348
50 µM – 250 µM	52.6475	*10.5348
50 µM – 500 µM	52.2775	*10.5348
100 µM – 250 µM	23.8050	*10.5348
100 µM – 500 µM	23.4350	*10.5348
250 µM – 500 µM	-0.3700	10.5348

* denotes a statistically significant difference

Table 25: ANOVA: Effect of H_2O_2 on cell viability after 8 hr incubation (between concentrations)

Source	Sum of squares	d.f	Mean square	F-ratio	Significance level
Between groups	53799.8	8	6724.97	149.85	0.0000*
Within groups	1211.69	27	44.8773		
Total	55011.5	35			

* $p < 0.05$

Table 26: Multiple range tests: Effect of H_2O_2 on cell viability after 8 hr incubation (between concentrations)

Concentrations	Difference	Limits
Control – 0.5 µM	0.6800	9.7194
Control – 1 µM	11.0425	*9.7194
Control – 5 µM	-7.3250	9.7194
Control – 10 µM	-4.3950	9.7194
Control – 50 µM	7.7975	9.7194
Control – 100 µM	64.2625	*9.7194
Control – 250 µM	89.5875	*9.7194
Control – 500 µM	89.7950	*9.7194

Concentrations	Difference	Limits
0.5 µM – 1 µM	10.3625	*9.7194
0.5 µM – 5 µM	-8.0050	9.7194
0.5 µM – 10 µM	-5.0750	9.7194
0.5 µM – 50 µM	7.1175	9.7194
0.5 µM – 100 µM	63.5825	*9.7194
0.5 µM – 250 µM	88.9075	*9.7194
0.5 µM – 500 µM	89.1150	*9.7194
1 µM – 5 µM	-18.3675	*9.7194
1 µM – 10 µM	-15.4375	*9.7194
1 µM – 50 µM	-3.2450	9.7194
1 µM – 100 µM	53.2200	*9.7194
1 µM – 250 µM	78.5450	*9.7194
1 µM – 500 µM	78.7525	*9.7194
5 µM – 10 µM	2.9300	9.7194
5 µM – 50 µM	15.1225	*9.7194
5 µM – 100 µM	71.5875	*9.7194
5 µM – 250 µM	96.9125	*9.7194
5 µM – 500 µM	97.1200	*9.7194
10 µM – 50 µM	12.1925	*9.7194
10 µM – 100 µM	68.6575	*9.7194
10 µM – 250 µM	93.9825	*9.7194
10 µM – 500 µM	94.1900	*9.7194
500 µM – 100 µM	56.4650	*9.7194
50 µM – 250 µM	81.7900	*9.7194
50 µM – 500 µM	81.9975	*9.7194
100 µM – 250 µM	25.3250	*9.7194
100 µM – 500 µM	25.5325	*9.7194
250 µM – 500 µM	0.2075	9.7194

* denotes a statistically significant difference

Table 27: ANOVA: Effect of H_2O_2 on cell viability after 12 hr incubation (between concentrations)

Source	Sum of squares	d.f	Mean square	F-ratio	Significance level
Between groups	62415.7	8	7801.96	164.01	0.0000*
Within groups	1284.39	27	47.5698		
Total	63700.1	35			

* $p < 0.05$

Table 28: Multiple range tests: Effect of H_2O_2 on cell viability after 12 hr incubation (between concentrations)

Concentrations	Difference	Limits
Control – 0.5 µM	4.0900	10.0068
Control – 1 µM	1.6175	10.0068
Control – 5 µM	5.0725	10.0068
Control – 10 µM	-2.8550	10.0068
Control – 50 µM	33.7450	*10.0068
Control – 100 µM	80.0175	*10.0068
Control – 250 µM	97.9100	*10.0068
Control – 500 µM	98.2100	*10.0068
0.5 µM – 1 µM	-2.4725	10.0068

Concentrations	Difference	Limits
0.5 µM – 5 µM	0.9825	10.0068
0.5 µM – 10 µM	-6.9450	10.0068
0.5 µM – 50 µM	29.6550	*10.0068
0.5 µM – 100 µM	75.9275	*10.0068
0.5 µM – 250 µM	93.8200	*10.0068
0.5 µM – 500 µM	94.1200	*10.0068
1 µM – 5 µM	3.4550	10.0068
1 µM – 10 µM	-4.4725	10.0068
1 µM – 50 µM	32.1275	*10.0068
1 µM – 100 µM	78.4000	*10.0068
1 µM – 250 µM	96.2925	*10.0068
1 µM – 500 µM	96.5925	*10.0068
5 µM – 10 µM	-7.9275	10.0068
5 µM – 50 µM	28.6725	*10.0068
5 µM – 100 µM	74.9450	*10.0068
5 µM – 250 µM	92.8375	*10.0068
5 µM – 500 µM	93.1375	*10.0068
10 µM – 50 µM	36.6000	*10.0068
10 µM – 100 µM	82.8725	*10.0068
10 µM – 250 µM	100.7650	*10.0068
10 µM – 500 µM	101.0650	*10.0068
500 µM – 100 µM	46.2725	*10.0068
50 µM – 250 µM	64.1650	*10.0068
50 µM – 500 µM	64.4650	*10.0068
100 µM – 250 µM	17.8925	*10.0068
100 µM – 500 µM	18.1925	*10.0068
250 µM – 500 µM	0.3000	10.0068

* denotes a statistically significant difference

Table 29: ANOVA: Effect of H_2O_2 on cell viability after 24 hr incubation (between concentrations)

Source	Sum of squares	d.f	Mean square	F-ratio	Significance level
Between groups	65341.8	8	8167.73	556.23	0.0000*
Within groups	396.469	27	14.684		
Total	65738.3	35			

* $p < 0.05$

Table 30: Multiple range tests: Effect of H_2O_2 on cell viability after 24 hr incubation (between concentrations)

Concentrations	Difference	Limits
Control – 0.5 µM	5.2625	5.5597
Control – 1 µM	2.8175	5.5597
Control – 5 µM	0.8250	5.5597
Control – 10 µM	4.9700	5.5597
Control – 50 µM	63.0175	*5.5597
Control – 100 µM	86.9550	*5.5597
Control – 250 µM	97.5825	*5.5597
Control – 500 µM	97.7425	*5.5597
0.5 µM – 1 µM	-2.4450	5.5597
0.5 µM – 5 µM	-4.4375	5.5597

Concentrations	Difference	Limits
0.5 µM – 10 µM	-0.2925	5.5597
0.5 µM – 50 µM	57.7550	*5.5597
0.5 µM – 100 µM	81.6925	*5.5597
0.5 µM – 250 µM	92.3200	*5.5597
0.5 µM – 500 µM	92.4800	*5.5597
1 µM – 5 µM	-1.9925	5.5597
1 µM – 10 µM	2.1525	5.5597
1 µM – 50 µM	60.2000	*5.5597
1 µM – 100 µM	84.1375	*5.5597
1 µM – 250 µM	94.7650	*5.5597
1 µM – 500 µM	94.9250	*5.5597
5 µM – 10 µM	4.1450	5.5597
5 µM – 50 µM	62.1925	*5.5597
5 µM – 100 µM	86.1300	*5.5597
5 µM – 250 µM	96.7575	*5.5597
5 µM – 500 µM	96.9175	*5.5597
10 µM – 50 µM	58.0475	*5.5597
10 µM – 100 µM	81.9850	*5.5597
10 µM – 250 µM	92.6125	*5.5597
10 µM – 500 µM	92.7725	*5.5597
500 µM – 100 µM	23.9375	*5.5597
50 µM – 250 µM	34.5650	*5.5597
50 µM – 500 µM	34.7250	*5.5597
100 µM – 250 µM	10.6275	*5.5597
100 µM – 500 µM	10.7875	*5.5597
250 µM – 500 µM	0.1600	5.5597

* denotes a statistically significant difference

Table 31: ANOVA: Cell viability of NG108-15 cells pre-treated with *H.erinaceus* extract (2 hr) followed by oxidative stress in MTT assay (between concentrations)

Source	Sum of squares	d.f	Mean square	F-ratio	Significance level
Between groups	11859.9	7	1694.28	63.78	0.0000*
Within groups	1700.06	64	26.5634		
Total	13560.0	71			

* $p < 0.05$

Table 32: Multiple range tests: Cell viability of NG108-15 cells pre-treated with *H.erinaceus* extract (2 hr) followed by oxidative stress in MTT assay (between concentrations)

Concentrations	Difference	Limits
Control – 0 µg/mL	38.9611	*4.8537
Control – 1 µg/mL	36.5167	*4.8537
Control – 10 µg/mL	37.5689	*4.8537
Control – 50 µg/mL	37.7967	*4.8537
Control – 100 µg/mL	39.2300	*4.8537
Control – 500 µg/mL	42.0033	*4.8537
Control – 1000 µg/mL	37.5978	*4.8537
0 µg/mL – 1 µg/mL	-2.4444	4.8537
0 µg/mL – 10 µg/mL	-1.3922	4.8537
0 µg/mL – 50 µg/mL	-1.1644	4.8537

Concentrations	Difference	Limits
0 µg/mL – 100 µg/mL	0.2689	4.8537
0 µg/mL – 500 µg/mL	3.0422	4.8537
0 µg/mL – 1000 µg/mL	-1.3633	4.8537
1 µg/mL – 10 µg/mL	1.0522	4.8537
1 µg/mL – 50 µg/mL	1.2800	4.8537
1 µg/mL – 100 µg/mL	2.7133	4.8537
1 µg/mL – 500 µg/mL	5.4867	*4.8537
1 µg/mL – 1000 µg/mL	1.0811	4.8537
10 µg/mL – 50 µg/mL	0.2278	4.8537
10 µg/mL – 100 µg/mL	1.6611	4.8537
10 µg/mL – 500 µg/mL	4.4344	4.8537
10 µg/mL – 1000 µg/mL	0.0289	4.8537
50 µg/mL – 100 µg/mL	1.4333	4.8537
50 µg/mL – 500 µg/mL	4.2067	4.8537
50 µg/mL – 1000 µg/mL	-0.1989	4.8537
100 µg/mL – 500 µg/mL	2.7733	4.8537
100 µg/mL – 1000 µg/mL	-1.6322	4.8537
500 µg/mL – 1000 µg/mL	-4.4056	4.8537

* denotes a statistically significant difference

Table 33: ANOVA: Cell viability of NG108-15 cells pre-treated with *H.erinaceus* extract (24 hr) followed by oxidative stress in MTT assay (between concentrations)

Source	Sum of squares	d.f	Mean square	F-ratio	Significance level
Between groups	22336.0	7	3190.85	85.06	0.0000*
Within groups	2400.91	64	37.5142		
Total	24736.9	71			

* $p < 0.05$

Table 34: Multiple range tests: Cell viability of NG108-15 cells pre-treated with *H.erinaceus* extract (24 hr) followed by oxidative stress in MTT assay (between concentrations)

Concentrations	Difference	Limits
Control – 0 µg/mL	43.6033	*5.7681
Control – 1 µg/mL	38.8000	*5.7681
Control – 10 µg/mL	44.0767	*5.7681
Control – 50 µg/mL	39.3444	*5.7681
Control – 100 µg/mL	45.7478	*5.7681
Control – 500 µg/mL	58.7089	*5.7681
Control – 1000 µg/mL	62.0511	*5.7681
0 µg/mL – 1 µg/mL	-4.8033	5.7681
0 µg/mL – 10 µg/mL	0.4733	5.7681
0 µg/mL – 50 µg/mL	-4.2589	5.7681
0 µg/mL – 100 µg/mL	2.1444	5.7681
0 µg/mL – 500 µg/mL	15.1056	*5.7681
0 µg/mL – 1000 µg/mL	18.4478	*5.7681
1 µg/mL – 10 µg/mL	5.2767	5.7681
1 µg/mL – 50 µg/mL	0.5444	5.7681
1 µg/mL – 100 µg/mL	6.9478	*5.7681
1 µg/mL – 500 µg/mL	19.9089	*5.7681
1 µg/mL – 1000 µg/mL	23.2511	*5.7681
10 µg/mL – 50 µg/mL	-4.7322	5.7681

Concentrations	Difference	Limits
10 µg/mL – 100 µg/mL	1.6711	5.7681
10 µg/mL – 500 µg/mL	14.6322	*5.7681
10 µg/mL – 1000 µg/mL	17.9744	*5.7681
50 µg/mL – 100 µg/mL	6.4033	*5.7681
50 µg/mL – 500 µg/mL	19.3644	*5.7681
50 µg/mL – 1000 µg/mL	22.7067	*5.7681
100 µg/mL – 500 µg/mL	12.9611	*5.7681
100 µg/mL – 1000 µg/mL	16.3033	*5.7681
500 µg/mL – 1000 µg/mL	3.3422	5.7681

* denotes a statistically significant difference

Table 35: ANOVA: Cell viability of NG108-15 cells co-treated with *H.erinaceus* extract and H_2O_2 (2 hr) in MTT assay (between concentrations)

Source	Sum of squares	d.f	Mean square	F-ratio	Significance level
Between groups	17243.4	7	2463.34	50.20	0.0000*
Within groups	3140.2	64	49.0657		
Total	20383.6	71			

* $p < 0.05$

Table 36: Multiple range tests: Cell viability of NG108-15 cells co-treated with *H.erinaceus* extract and H_2O_2 (2 hr) in MTT assay (between concentrations)

Concentrations	Difference	Limits
Control – 0 µg/mL	45.9756	*6.5966
Control – 1 µg/mL	43.3300	*6.5966
Control – 10 µg/mL	42.9756	*6.5966
Control – 50 µg/mL	45.0289	*6.5966
Control – 100 µg/mL	43.4400	*6.5966
Control – 500 µg/mL	49.9522	*6.5966
Control – 1000 µg/mL	51.1600	*6.5966
0 µg/mL – 1 µg/mL	-2.6456	6.5966
0 µg/mL – 10 µg/mL	-3.0000	6.5966
0 µg/mL – 50 µg/mL	-0.9467	6.5966
0 µg/mL – 100 µg/mL	-2.5356	6.5966
0 µg/mL – 500 µg/mL	3.9767	6.5966
0 µg/mL – 1000 µg/mL	5.1844	6.5966
1 µg/mL – 10 µg/mL	-0.3544	6.5966
1 µg/mL – 50 µg/mL	1.6989	6.5966
1 µg/mL – 100 µg/mL	0.1100	6.5966
1 µg/mL – 500 µg/mL	6.6222	*6.5966
1 µg/mL – 1000 µg/mL	7.8300	*6.5966
10 µg/mL – 50 µg/mL	2.0533	6.5966
10 µg/mL – 100 µg/mL	0.4644	6.5966
10 µg/mL – 500 µg/mL	6.9767	*6.5966
10 µg/mL – 1000 µg/mL	8.1844	*6.5966
50 µg/mL – 100 µg/mL	-1.5889	6.5966
50 µg/mL – 500 µg/mL	4.9233	6.5966
50 µg/mL – 1000 µg/mL	6.1311	6.5966
100 µg/mL – 500 µg/mL	6.5122	6.5966
100 µg/mL – 1000 µg/mL	7.7200	*6.5966
500 µg/mL – 1000 µg/mL	1.2078	6.5966

* denotes a statistically significant difference

Table 37: ANOVA: Cell viability of NG108-15 cells pre-treated with *H.erinaceus* extract (2 hr) followed by oxidative stress in trypan blue exclusion assay (between concentrations)

Source	Sum of squares	d.f	Mean square	F-ratio	Significance level
Between groups	4467.94	7	638.278	25.67	0.0000*
Within groups	596.713	24	24.863		
Total	5064.66	31			

* $p < 0.05$

Table 38: Multiple range tests: Cell viability of NG108-15 cells pre-treated with *H.erinaceus* extract (2 hr) followed by oxidative stress in trypan blue exclusion assay (between concentrations)

Concentrations	Difference	Limits
Control – 0 µg/mL	34.8625	*7.27698
Control – 1 µg/mL	31.7475	*7.27698
Control – 10 µg/mL	36.3650	*7.27698
Control – 50 µg/mL	32.3650	*7.27698
Control – 100 µg/mL	33.7175	*7.27698
Control – 500 µg/mL	31.8750	*7.27698
Control – 1000 µg/mL	41.2900	*7.27698
0 µg/mL – 1 µg/mL	-3.1150	7.27698
0 µg/mL – 10 µg/mL	1.5025	7.27698
0 µg/mL – 50 µg/mL	-2.4975	7.27698
0 µg/mL – 100 µg/mL	-1.1450	7.27698
0 µg/mL – 500 µg/mL	-2.9875	7.27698
0 µg/mL – 1000 µg/mL	6.4275	7.27698
1 µg/mL – 10 µg/mL	4.6175	7.27698
1 µg/mL – 50 µg/mL	0.6175	7.27698
1 µg/mL – 100 µg/mL	1.9700	7.27698
1 µg/mL – 500 µg/mL	0.1275	7.27698
1 µg/mL – 1000 µg/mL	9.5425	*7.27698
10 µg/mL – 50 µg/mL	-4.0000	7.27698
10 µg/mL – 100 µg/mL	-2.6475	7.27698
10 µg/mL – 500 µg/mL	-4.4900	7.27698
10 µg/mL – 1000 µg/mL	4.9250	7.27698
50 µg/mL – 100 µg/mL	1.3525	7.27698
50 µg/mL – 500 µg/mL	-0.4900	7.27698
50 µg/mL – 1000 µg/mL	8.9250	*7.27698
100 µg/mL – 500 µg/mL	-1.8425	7.27698
100 µg/mL – 1000 µg/mL	7.5725	*7.27698
500 µg/mL – 1000 µg/mL	9.4150	*7.27698

* denotes a statistically significant difference

Table 39: ANOVA: Cell viability of NG108-15 cells pre-treated with *H.erinaceus* extract (24 hr) followed by oxidative stress in trypan blue exclusion assay (between concentrations)

Source	Sum of squares	d.f	Mean square	F-ratio	Significance level
Between groups	1842.19	7	263.171	23.48	0.0000*
Within groups	268.976	24	11.2073		
Total	2111.17	31			

* $p < 0.05$

Table 40: Multiple range tests: Cell viability of NG108-15 cells pre-treated with *H.erinaceus* extract (24 hr) followed by oxidative stress in trypan blue exclusion assay (between concentrations)

Concentrations	Difference	Limits
Control – 0 µg/mL	28.8325	*4.8857
Control – 1 µg/mL	14.9250	*4.8857
Control – 10 µg/mL	11.1900	*4.8857
Control – 50 µg/mL	15.7975	*4.8857
Control – 100 µg/mL	9.8275	*4.8857
Control – 500 µg/mL	17.7300	*4.8857
Control – 1000 µg/mL	15.2400	*4.8857
0 µg/mL – 1 µg/mL	-13.9075	*4.8857
0 µg/mL – 10 µg/mL	-17.6425	*4.8857
0 µg/mL – 50 µg/mL	-13.0350	*4.8857
0 µg/mL – 100 µg/mL	-19.0050	*4.8857
0 µg/mL – 500 µg/mL	-11.1025	*4.8857
0 µg/mL – 1000 µg/mL	-13.5925	*4.8857
1 µg/mL – 10 µg/mL	-3.7350	4.8857
1 µg/mL – 50 µg/mL	0.8725	4.8857
1 µg/mL – 100 µg/mL	-5.0975	*4.8857
1 µg/mL – 500 µg/mL	2.8050	4.8857
1 µg/mL – 1000 µg/mL	0.3150	4.8857
10 µg/mL – 50 µg/mL	4.6075	4.8857
10 µg/mL – 100 µg/mL	-1.3625	4.8857
10 µg/mL – 500 µg/mL	6.5400	*4.8857
10 µg/mL – 1000 µg/mL	4.0500	4.8857
50 µg/mL – 100 µg/mL	-5.9700	*4.8857
50 µg/mL – 500 µg/mL	1.9325	4.8857
50 µg/mL – 1000 µg/mL	-0.5575	4.8857
100 µg/mL – 500 µg/mL	7.9025	*4.8857
100 µg/mL – 1000 µg/mL	5.4125	*4.8857
500 µg/mL – 1000 µg/mL	-2.4900	4.8857

* denotes a statistically significant difference

Table 41: ANOVA: Cell viability of NG108-15 cells co-treated with *H.erinaceus* extract and H_2O_2 (2 hr) in trypan blue exclusion assay (between concentrations)

Source	Sum of squares	d.f	Mean square	F-ratio	Significance level
Between groups	2839.57	7	405.653	12.81	0.0000*
Within groups	759.85	24	31.6604		
Total	3599.42	31			

* $p < 0.05$

Table 42: Multiple range tests: Cell viability of NG108-15 cells co-treated with *H.erinaceus* extract and H_2O_2 (2 hr) in trypan blue exclusion assay (between concentrations)

Concentrations	Difference	Limits
Control – 0 µg/mL	23.9450	*8.2117
Control – 1 µg/mL	21.6750	*8.2117
Control – 10 µg/mL	23.2850	*8.2117
Control – 50 µg/mL	24.6875	*8.2117
Control – 100 µg/mL	28.6100	*8.2117
Control – 500 µg/mL	28.9375	*8.2117

Concentrations	Difference	Limits
Control – 1000 µg/mL	33.4225	*8.2117
0 µg/mL – 1 µg/mL	-2.2700	8.2117
0 µg/mL – 10 µg/mL	-0.6600	8.2117
0 µg/mL – 50 µg/mL	0.7425	8.2117
0 µg/mL – 100 µg/mL	4.6650	8.2117
0 µg/mL – 500 µg/mL	4.9925	8.2117
0 µg/mL – 1000 µg/mL	9.4775	*8.2117
1 µg/mL – 10 µg/mL	1.6100	8.2117
1 µg/mL – 50 µg/mL	3.0125	8.2117
1 µg/mL – 100 µg/mL	6.9350	8.2117
1 µg/mL – 500 µg/mL	7.2625	8.2117
1 µg/mL – 1000 µg/mL	11.7475	*8.2117
10 µg/mL – 50 µg/mL	1.4025	8.2117
10 µg/mL – 100 µg/mL	5.3250	8.2117
10 µg/mL – 500 µg/mL	5.6525	8.2117
10 µg/mL – 1000 µg/mL	10.1375	*8.2117
50 µg/mL – 100 µg/mL	3.9225	8.2117
50 µg/mL – 500 µg/mL	4.2500	8.2117
50 µg/mL – 1000 µg/mL	8.7350	*8.2117
100 µg/mL – 500 µg/mL	0.3275	8.2117
100 µg/mL – 1000 µg/mL	4.8125	8.2117
500 µg/mL – 1000 µg/mL	4.4850	8.2117

* denotes a statistically significant difference

Table 43: ANOVA: Evaluation of apoptotic cells in NG108-15 cells subjected to different treatments using TUNEL assay (between treatments)

Source	Sum of squares	d.f	Mean square	F-ratio	Significance level
Between groups	579.654	3	193.218	15.66	0.0002*
Within groups	148.08	12	12.34		
Total	727.734	15			

* $p < 0.05$

Table 44: Multiple range tests: Evaluation of apoptotic cells in NG108-15 cells subjected to different treatments using TUNEL assay (between treatments)

Treatments	Difference	Limits
Control – 100 µg/mL extract	-9.7825	*5.4121
Control – 100 µM H_2O_2	-15.5700	*5.4121
Control – extract/H_2O_2	-13.7475	*5.4121
100 µg/mL extract – 100 µM H_2O_2	-5.7875	*5.4121
100 µg/mL extract – extract/H_2O_2	-3.9650	5.4121
100 µM H_2O_2 – extract/H_2O_2	1.8225	5.4121