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Application of Artificial Neural Network for Modeling and Prediction of MTT Assay on Human Lung Epithelial Cancer Cell Lines

Taghipour M[1,2], Vand AA[2], Rezaei A[3] and Karim GR[4]*

[1]Department of Biomedical Engineering, Faculty of medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran
[2]Department of Computer Engineering, Islamic Azad University, Kermanshah, Iran
[3]Eelectrical Faculty, Kermanshah University of Technology, Kermanshah, Iran
[4]Department of Electrical Engineering, Razi University, Kermanshah, Iran

Abstract

In this paper, a three-layer artificial neural network (ANN) was investigated to predict the inhibitory concentration (IC) values assessed via MTT cell viability assay on the four types of human lung epithelial cancer cell lines. In order to achieve this purpose, a multilayer perceptron (MLP) neural network trained with back-propagation algorithm was employed for developing the ANN model. To develop the model, the input parameters were concentrations and types of cell lines and the outputs were IC10, IC20, IC30, IC40, IC50, IC60, IC70 and IC80 values in the A549, H157, H460 and H1975 cell lines. The proposed ANN model has achieved good agreement with the experimental data and has a small error between the estimated and experimental values. The obtained results show that the proposed ANN model is a useful, reliable, fast and cheap tool to predict the IC values assessed via MTT cell viability assays.

Keywords: MTT assay; Artificial neural network; Multilayer perceptron; Modeling; Inhibitory concentration

Abbreviations: ANN: Artificial Neural Network; RBF: Radial Basis Function; ANOVA: Analysis of Variance ; CF: Correlation Factor; CO_2: Carbon Dioxide; DMSO: Dimethyl Sulfoxide; DOX: Doxorubicin; FBS: Fetal Bovine Serum; IC: Inhibitory Concentration; IC50: Inhibitory Concentration of 50% Of Enzyme Activity; MAE: Mean Absolute Error; MRE: Mean Relative Error; MTT: 3-(4:5-Dimethyl-2-Thiazol)-2:5-Diphenyl-2H-Tetrazolium Bromide; MLP: Multilayer Perceptron; RMSE: Root Mean Square Error; RPMI: Roswell Park Memorial Institute Medium

Introduction

Many biological assays require the measurement of surviving and/or proliferating mammalian cells. This can be achieved by several methods, e.g., counting cells that include/exclude a dye, measuring released ^{51}Cr-labeled protein after cell lysis, and measuring incorporation of radioactive nucleotides ([^{3}H]thymidineor [^{125}I] iodo-deoxyuridine) during cell proliferation [1]. In 1983, a new and rapid quantitative colorimetric assay, based on the tetrazolium salt thiazolyl blue, for mammalian cell survival and cell proliferation was proposed by Mosmann [1]. At present, colorimetric assay using the tetrazolium salt thiazolyl blue, also termed MTT, after methyl-thiazolyl-tetrazolium [2] is widely used for assessment of cytotoxicity, cell viability and proliferation studies in cell biology [2-5]. This test is based on the cellular uptake of MTT and its subsequent reduction in the mitochondria of living cells to MTT formazan (a dark, water insoluble and blue product) [6]. The method has been extended and improved by several authors [7-10].

Doxorubicin (DOX, trade name Adriamycin) is an antitumor drug commonly used as single and in combination with other chemo-therapeutic agents, for treatment of wide range of human malignancies [11,12].

Flavonoids have been extensively studied for their multifaceted ability to perform as chemoprevention agents and as chemotherapeutics against a wide array of cancers [13,14]. Chrysin is aflavone found in the blue passion flower (Passifloracaerulea) and Honeycomb [13,15].

Artificial neural network (ANN) is a highly simplified model of the biological network structure[16,17]. The basic advantage of ANN is that it does not need any mathematical model; an ANN learns from examples and recognizes patterns in a series of input and output data without any prior assumptions about their nature and interrelations [17]. Moreover, ANN is a good alternative to conventional empirical modeling based on polynomial and linear regressions [18]. Thus, ANN is a typical non-mechanistic model for modeling complex information and is known to have two intrinsic advantages. The first is its flexible capacity in apprehending the data used for training. Being intrinsically nonlinear, a trained ANN can grasp certain subtle patterns that tend to be overlooked by common statistical methods. The second advantage is its high predictive accuracy, i.e., the predictive capability for "new" data (untrained data) [19-23]. The high predictive accuracy is an assured outcome of the ability of ANN to apprehend the data [21,24,25]. On recognizing and application these advantages of ANN in MTT assays, in the current study, we report the design, training and validation of a feed-forward ANN to predict the inhibitory concentration (IC) data such that the designed ANN would (A) make sufficient use of the existing ICs data table of an available set of experimental data about chrysin enhances doxorubicin-induced cytotoxicity in human lung epithelial cancer cell lines by Brechbuhl et al. [13], (B) predict the ICs evaluated with a MTT assay in human lung epithelial cancer cell lines treated with chrysin before exposure to DOX. The predicted ICs are expected to fill the data gap for untested IC values with less waste of time and resources.

***Corresponding author:** Karim GR, Department of Electrical Engineering, Razi University, Kermanshah, Iran, Tel: +98 0918237 9045; Fax: +98 831 427 4623; E-mail: ghkarimi@razi.ac.ir

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Materials and Methods

Compiling MTT assay data for the ANN model

Materials, cell culture and MTT assay conditions: For training the ANN model, we used experimental data evaluated by Brechbuhl [13]. The cytotoxic effects of combination drug therapy with chrysin and DOX were determined against cell lines using MTT [1]. The lung non-small cell epithelial cancer cell lines A549, H157, H460 and H1975 were cultured at 37°C with 5% CO_2 and grown in media and supplements purchased from Mediatech (Manassas, VA). All cells were grown in RPMI media containing L-glutamine and supplemented with 10% FBS (Gemini Bio-Products, West Sacramento, CA). Cells were maintained in T-150 flasks and split into 96-well plates at least 18 h prior to treatment. H157 and A549 cells were seeded for treatment at 12,000 cells per well. H460 and H1975 cells were seeded for treatment at 10,000 cells per well. At the time of treatment all wells were approximately 70-75% confluent and were treated with fresh media containing the indicated compounds. After exposure, 20 µl/well of MTT solution (5 mg/ml phosphate buffered saline) was added and incubated for 3-4 h. The medium was aspirated and replaced with 150 µl/well DMSO to dissolve the formazan salt. The color intensity of the formazan solution, which reflects the cell growth condition, was measured at 570 nm using a Spectra Max 340PC plate reader (Molecular Devices, Sunnyvale, CA).

Statistical analysis of the MTT assay data: Experimental data evaluated by Brechbuhl [13] were expressed as the mean ± standard error of the mean (S.E.M.). All experiments included at least triplicate treatment groups and each experiment was repeated at least two times. ANOVA comparison, Tukey comparison, t-tests, linear regression curves and cytotoxicities (ICs) were calculated using Prism version 5 (GraphPad, San Diego, CA).

Modeling Approach

Artificial neural network

Artificial neural networks (ANN) is a good technique used to handle problems of modeling, prediction, control and classification [22]. An ANN is based on the operation of biological neural networks. The fundamental processing element of ANN is an artificial neuron (or simply a neuron). A biological neuron receives inputs from other sources, combines them, performs generally a nonlinear operation on the result, and then outputs the final result [20,23,25]. ANNs have been used in many different applications such as finance, medicine, engineering, geology and physics [19,22,23]. ANN eliminates the limitations of the classical approaches by extracting the desired information using the input data. Applying ANN to a system needs sufficient input and output data instead of a mathematical equation [24-27]. Multilayer perceptron (MLP) networks are the most widely used neural networks that consist of a great number of processing elements called neurons. An MLP network has one input layer, one or more hidden layer and one output layer as shown in Figure 1.

The output from q[th] neuron of the first hidden layer is given by [20]:

$$\beta_q = f(\sum_{k=1}^{p}(x_k W_{kq}) + b_q) \qquad q = 1,2,....,Q \tag{1}$$

Where x is the inputs, Q is the number of neurons in the first hidden layer, p is the number of neurons in the input layer, b is the bias term, W is the weighting factor and f is the activation function of the first hidden layer. The output of the m[th] neuron in the output layer is given by:

$$y_m = \sum_{k=1}^{s}(\theta_k W_{km}) + b_m \qquad m = 1,2,....,M \tag{2}$$

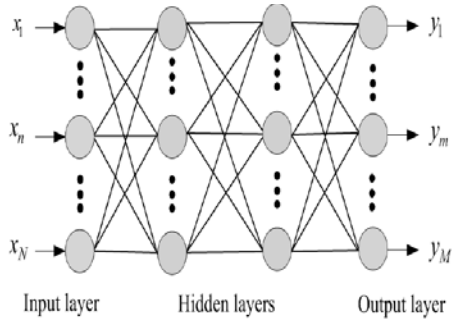

Figure 1: MLP structure.

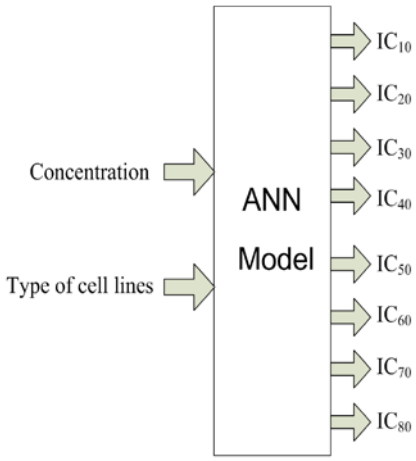

Figure 2: Simplified overview of the proposed ANN model.

Where b is the bias term, W is weighting factor, s is the number of neurons in the second hidden layer, M is the number of neurons in the output layer.

Developing the model

The simplified overview of the proposed MLP model is shown in Figure 2, where the inputs are concentrations and types of cell lines and the outputs are IC_{10}, IC_{20}, IC_{30}, IC_{40}, IC_{50}, IC_{60}, IC_{70} and IC_{80} values in the A549, H157, H460 and H1975 cell lines. The data set required for training the network is obtained using the experimental values [13]. For developing the ANN model, the experimental data are divided into two sets. The number of samples for training and testing are 21 (about 75%) and 7(about 25%).

In this study, different ANN structures were tested and optimized to obtain the best ANN configuration. We tested many different structures with one, two, three and four hidden layers with different number of neurons in each layer also we tested Radial basis function (RBF) for prediction output. Table 1 show the comparison between these structures, where the mean relative error percentage (MRE %) is given by:

$$MRE\% = 100 \times \frac{1}{N}\sum_{i=1}^{N}\left|\frac{X_i(Exp) - X_i(\Pr ed)}{X_i(Exp)}\right| \tag{3}$$

Where N is the number of data and '*X(Exp)*' and '*X (Pred)*' stands for experimental and predicted (ANN) values respectively.

Also we tested many ANN configurations with different structure, different training algorithm and different number of epochs. Table 2 shows the obtained MRE% for these different ANN configurations. The best obtained ANN structure in Table 1 is the latest ANN structure in Table 2.

As it is shown in Tables 1 and 2, the MLP model with 2-11-8-9-8 structure (i.e., 2 neurons in the input layer, 11 neurons in the first hidden layer, 8 neurons in the second hidden layer, 9 neurons in the third hidden layer and 8 neurons in the output layer) has the least MRE%. Therefore, we selected this structure in this paper.

Results and Discussions

Table 3 shows the specification of the proposed ANN model. The training and testing results for the proposed ANN model in comparison with experimental results [13] are shown in Tables 4 and 5 respectively.

Table 6 shows the obtained errors for the proposed ANN model, where the mean absolute error percentage (MAE %), the root mean square error (RMSE), and the correlation factor (CF) of the proposed ANN models are calculated by:

ANN Structure	Average of MRE%	
	Train	Test
2-8-11-8	1.203	15.375
2-12-10-8	4.635	22.25
2-7-8-8	6.505	21.625
2-9-8-8	8.95	23.5
2-11-8-9-8	**0.311**	**11.375**
2-10-8-11-8	2.78	16.05
2-7-12-7-8	3.437	16.5
2-7-10-12-8	2.020	14.5
2-9-7-11-8	7.046	14.625
RBF	1.977e-13	61.323

Table 1: Average of MRE% for all outputs for different ANN structures.

$$MAE\% = 100 \times \frac{1}{N} \sum_{i=1}^{Z} \left| X_i(Exp) - X_i(\Pr ed) \right| \quad (7)$$

$$RMSE = \left[\frac{\sum_{i=1}^{N} (X_i(Exp) - X_i(\Pr ed))^2}{N} \right]^{0.5} \quad (8)$$

$$CF = 1 - \left[\frac{\sum_{i=1}^{N} (X_i(Exp) - X_i(\Pr ed))^2}{\sum_{i=1}^{N} (X_i(Exp))^2} \right] \quad (9)$$

Where N is the number of data and '*X(Exp)*' and '*X(Pred)*' stand for experimental and predicted (ANN) values respectively. Figure 3 shows the comparison between the experimental and predicted results using ANN for IC_{50} for all data in A549, H157, H460 and H1975 cells.

Figure 4 shows the comparison between the experimental and predicted results using ANN for IC_{50} for all data in A549, H157, H460 and H1975 cell lines. The result shows that there is a good agreement between the experimental and predicted values for output parameters and the ANN model can be used as an accurate model to prediction of inhibitory concentration values accessed via MTT assay on human lung epithelial cancer cell lines co-treated with chrysin and doxorubicin.

Conclusion

In this paper, the inhibitory concentration (IC) values assessed via MTT cell viability assay on the four types of human lung epithelial cancer cell lines is modeled and predicted by artificial neural network. The proposed ANN model has achieved good agreement with the experimental data with minimum error. According to the obtained results from the ANN model and comparing them with the experimental results, it can be shown that ANN can be used in modeling and output prediction of the IC values assessed via MTT cell viability assays. Seems that the biggest achievement of the Modeling and prediction of the inhibitory concentration values assessed via MTT

Algorithm	Number of hidden layer	Epoch	The obtained MRE% for each output (for testing data)								Average of MRE% for all outputs (for testing data)	Average of MRE% for all outputs (for training data)
			Ic10	Ic20	Ic30	Ic40	Ic50	Ic60	Ic70	Ic80		
trainbr	2	400	27.0151	17.9206	16.7244	12.0143	13.0745407	13.3276	14.1787	17.6571	16.48	12.16
trainbr	2	700	22.3788	20.0544	16.5557	15.6283	16.7017502	10.9127	17.2621	16.4354	16.99	0.3
trainbr	1	500	21.048	16.0378	17.6698	15.5808	18.6307329	14.1561	20.4347	22.0517	18.2	10.68
trainbr	1	1300	33.8414	21.0104	18.3558	12.529	15.1277031	18.2815	17.0795	19.6552	19.48	9.44
trainoss	1	700	20.7053	14.4374	15.1151	13.5781	14.2723244	17.5177	20.7295	23.9746	17.54	12.59
trainoss	1	1350	20.2299	20.2299	20.2299	20.2299	20.2299053	20.2299	20.2299	20.2299	20.22	14.21
trainoss	2	300	23.0166	17.9729	18.5065	15.644	13.347814	12.675	20.2906	24.0792	18.19	11.08
trainoss	2	800	27.17	24.0429	22.0162	19.1207	19.0589384	19.9404	16.5142	19.2009	20.88	14.5
trainrp	1	800	22.8237	18.1264	15.4406	12.8661	13.3224577	9.71382	12.1282	12.817	14.5	13.74
trainrp	1	1400	21.3078	16.2821	18.6634	17.2872	19.5747347	12.6446	21.9342	23.6726	18.92	10.46
trainrp	2	600	23.183	19.7428	22.5653	17.6145	19.198957	9.78751	20.6894	18.6031	18.923	10.62
trainrp	2	1000	36.9176	25.7577	22.384	16.2173	16.4267443	14.7662	15.7739	18.5915	20.854	9.47
trainlm	1	700	17.5779	15.7346	16.2147	14.6758	15.2469949	18.1365	15.1962	15.5707	16.04	9.29
trainlm	1	1400	33.8414	21.0104	18.3558	12.529	15.1277031	18.2815	17.0795	19.6552	19.48	9.44
trainlm	2	600	35.5098	40.2898	41.9465	43.7499	40.9964492	37.1257	48.6457	55.9676	43.028	0.022
trainlm	2	400	19.9041	19.6094	21.8283	21.7855	24.9685346	25.5053	29.3438	33.3467	24.536	0.45
trainlm	3	1000	14.13	12.28	8.3911	9.562	7.1886	11.57	13.4965	14.4031	11.375	0.311

Table 2: Comparison of the ANN configurations with different training algorithm, number of hidden layers and number of epochs.

Neural network	MLP
Number of hidden layer	3
Number of neurons in the input layer	2
Number of neurons in the first hidden layer	11
Number of neurons in the second hidden layer	8
Number of neurons in the third hidden layer	9
Number of neurons in the output layer	8
Learning rate	0.5
Number of epochs	1000
Adaption learning function	trainlm
Activation function	tansig

Table 3: Comparison of the ANN configurations with different training algorithms, number of hidden layers and number of epochs.

Type of cell lines	Concentration	Experimental (Brechbuhl et al., 2012)								ANN							
		IC10	IC20	IC30	IC40	IC50	IC60	IC70	IC80	IC10	IC20	IC30	IC40	IC50	IC60	IC70	IC80
A549	0	0.042	0.093	0.158	0.245	0.365	0.543	0.839	1.43	0.041978	0.093261	0.157868	0.244424	0.364832	0.543638	0.838374	1.431309
A549	5	0.041	0.085	0.137	0.203	0.291	0.418	0.62	1	0.041035	0.084946	0.13698	0.202963	0.290897	0.417973	0.619953	1.000615
A549	10	0.04	0.081	0.128	0.187	0.264	0.374	0.545	0.864	0.040075	0.080859	0.128033	0.186818	0.263701	0.3739	0.546035	0.864042
A549	20	0.029	0.061	0.099	0.147	0.212	0.306	0.456	0.742	0.028952	0.060985	0.09902	0.147473	0.211931	0.306434	0.455652	0.740717
A549	25	0.018	0.046	0.085	0.14	0.223	0.354	0.587	1.087	0.018011	0.046008	0.084982	0.139996	0.222723	0.353856	0.587	1.088188
A549	30	0.017	0.045	0.086	0.146	0.238	0.388	0.661	1.267	0.016991	0.045034	0.085969	0.145809	0.238652	0.387638	0.661005	1.266474
H157	5	0.075	0.135	0.199	0.274	0.367	0.492	0.678	1	0.074995	0.134953	0.199222	0.274026	0.366762	0.491727	0.677891	1.000537
H157	10	0.095	0.153	0.212	0.275	0.35	0.446	0.581	0.801	0.094935	0.15301	0.212266	0.274863	0.350125	0.446003	0.58088	0.800786
H157	15	0.072	0.124	0.179	0.241	0.317	0.417	0.562	0.809	0.072014	0.123935	0.178989	0.241067	0.317033	0.417155	0.561825	0.808929
H157	25	0.062	0.114	0.171	0.239	0.325	0.442	0.671	0.928	0.061993	0.11399	0.171019	0.238955	0.325174	0.441993	0.670977	0.927794
H157	30	0.075	0.132	0.193	0.264	0.351	0.467	0.638	0.932	0.075	0.131999	0.192638	0.264524	0.351302	0.466654	0.638305	0.931391
H460	0	0.012	0.022	0.034	0.049	0.067	0.042	0.133	0.205	0.011998	0.021983	0.034004	0.049047	0.067017	0.042005	0.133019	0.204852
H460	10	0.011	0.017	0.023	0.028	0.035	0.039	0.053	0.07	0.011	0.01701	0.023007	0.02797	0.034991	0.038998	0.052994	0.070043
H460	15	0.013	0.017	0.021	0.025	0.03	0.02	0.042	0.051	0.013006	0.017006	0.020991	0.025007	0.029951	0.019998	0.042003	0.05104
H460	20	0.014	0.02	0.024	0.029	0.033	0.02	0.046	0.056	0.013996	0.020009	0.024003	0.028968	0.033037	0.019999	0.045991	0.056002
H460	30	0.003	0.006	0.008	0.011	0.015	0.02	0.027	0.039	0.003	0.005999	0.008001	0.011002	0.015005	0.020001	0.027	0.038988
H1975	0	0.014	0.025	0.036	0.05	0.066	0.087	0.119	0.173	0.014	0.024941	0.036074	0.050112	0.065883	0.087021	0.118999	0.172921
H1975	5	0.018	0.024	0.03	0.036	0.042	0.049	0.058	0.072	0.017998	0.024027	0.029924	0.035995	0.042096	0.048992	0.058014	0.071943
H1975	15	0.013	0.017	0.021	0.024	0.028	0.033	0.038	0.047	0.012995	0.016948	0.020973	0.024123	0.028047	0.033006	0.038029	0.046846
H1975	20	0.013	0.019	0.024	0.029	0.034	0.041	0.05	0.063	0.013008	0.019028	0.023958	0.028992	0.033963	0.040999	0.050002	0.063069
H1975	30	0.015	0.02	0.025	0.03	0.035	0.041	0.049	0.061	0.014999	0.020046	0.025096	0.029813	0.034954	0.040992	0.048949	0.061195

Table 4: Specification of the best proposed ANN model.

Type of cell lines	Concentration	Experimental (Brechbuhl et al., 2012)								ANN							
		IC10	IC20	IC30	IC40	IC50	IC60	IC70	IC80	IC10	IC20	IC30	IC40	IC50	IC60	IC70	IC80
A549	15	0.031	0.062	0.1	0.147	0.21	0.3	0.441	0.707	0.028841	0.061222	0.099951	0.14958	0.215928	0.313467	0.467635	0.7657
H157	0	0.065	0.147	0.253	0.393	0.589	0.884	1.38	2.36	0.082755	0.158206	0.245803	0.350962	0.490139	0.678978	0.967511	2.219227
H157	20	0.091	0.15	0.209	0.247	0.351	0.451	0.591	0.823	0.068937	0.123067	0.181563	0.250181	0.335246	0.449087	0.633137	0.91099
H460	5	0.01	0.016	0.021	0.026	0.033	0.035	0.052	0.07	0.008354	0.009992	0.016618	0.019554	0.030154	0.027742	0.055977	0.071152
H460	25	0.004	0.007	0.009	0.013	0.016	0.02	0.026	0.036	0.003458	0.006649	0.008694	0.011851	0.015852	0.020166	0.027423	0.039216
H1975	10	0.017	0.023	0.029	0.035	0.042	0.05	0.06	0.076	0.015019	0.019366	0.023818	0.027586	0.031505	0.032587	0.041633	0.051344
H1975	25	0.014	0.019	0.024	0.028	0.034	0.04	0.047	0.059	0.013679	0.019547	0.024476	0.029507	0.034269	0.039141	0.049207	0.062233

Table 5: The Comparison between experimental and predicted ANN results for training data.

assay using artificial neural network that was conducted in this paper is create a newer, faster and more efficient method with a very low cost and with high accuracy. The development of this method can be allows finding the optimal drug concentrations on different cell lines using computational intelligence modeling without repeating them in vitro.

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Authors' Contributions

Designing the method of study; collection, validation of the data: Mostafa Taghipour, Abbas Rezaei, analysis of the data; drafting the manuscript and final revision: Ayoub Adineh Vand. Validation and analysis of the data and final revision:

Output	MAE		RMSE		CF	
	Train	Test	Train	Test	Train	Test
IC10	1.52E-05	0.006638	2.62E-05	0.010782	0.999999	0.935947
IC20	4.28E-05	0.007065	7.21E-05	0.011347	0.999999	0.98275
IC30	7.02E-05	0.006433	0.000119	0.011026	0.999999	0.996787
IC40	0.000134	0.009188	0.000218	0.016406	0.999998	0.996212
IC50	0.000138	0.019186	0.000203	0.038126	0.999999	0.994751
IC60	0.000121	0.035157	0.000216	0.077989	0.999999	0.9862
IC70	0.000139	0.072462	0.000288	0.157204	0.999999	0.971827
IC80	0.000352	0.145674	0.000547	0.320428	0.999999	0.960799

Table 6: The Comparison between experimental and predicted ANN results for testing data.

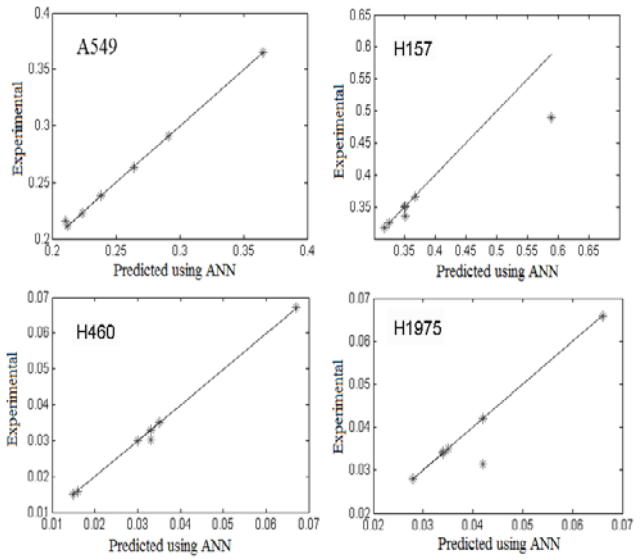

Figure 3: Comparison with the experimental for IC_{50} in A549, H157, H460 and H1975 cells.

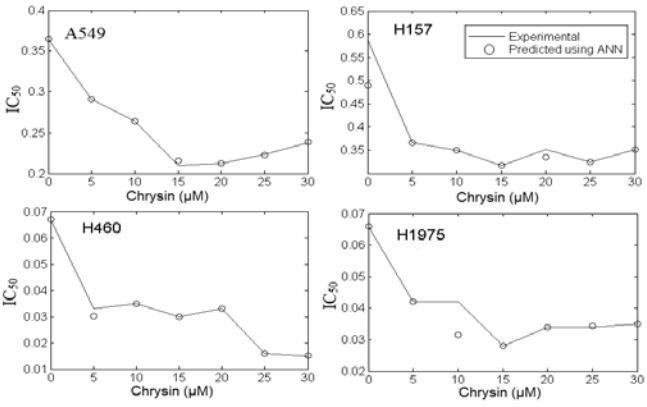

Figure 4: Comparison of the ANN and experimental results for IC_{50} as a function of Chrysin on DOX -induced cytotoxicity in A549, H157, H460 and H1975 cell lines.

G.H.Karimi, The authors declare their sincere gratification to the family and friends for their huge support during the time spent on this article.

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