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CYTOTOXICITY PROFILING OF METAL OXIDE NANOPARTICLES TOWARDS ESCHERICHIA COLI USING QSAR MODELING

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Abstract
Nanotechnology is quickly developing, manufacturing nanomaterials of different genera, of which, metal oxide nanoparticles, is the most demanding class of nanomaterials. Currently, the expedient heightening of metal oxide nanoparticle's usage poses an impact on the environment. As, nanotoxicity profile of the vast majority of these metal oxide nanoparticles is as yet unclear and the information with respect to their physicochemical properties that contributes towards their bioactivity is also scant. In addition, trial assessment of each and every current and recently integrated metal oxide nanoparticle is very costly, arduous and tedious. Likewise, low sufficiency of invivo/invitro experimental designs encumbers the toxicity evaluation of nanoparticles. Consequently, computational insilico QSAR (quantitative structure- activity relationship) approach have been investigated as successful technique for assessing the harmful cytotoxic effects of metal oxide nanoparticles in *Escherichia coli*. In addition, both the models were assessed for their prediction accuracy based on the merit of F-measure. In the current work, nano-QSAR models have been developed employing computed size autonomous nano-explicit descriptors using machine learning algorithm including linear multiple linear regression (MLR) and non-linear neural network (NN). Deciphered from the developed models, nanodescriptors including $\sum\chi$=nO, χox and ΔH_{Me+} effectively encodes the metal oxide nanoparticle's cytotoxicity mechanism in *Escherichia coli*. And furthermore, the value of F-measure for linear MLR based model was higher i.e., 85% than non-linear NN model i.e.,74% indicating improved prediction competence of linear MLR model over non-linear NN model in *Escherichia coli*. Thus, MLR based QSAR model displayed high statistical robustness in the proposed specie for toxicity profiling. Subsequently, this study underscores on the importance of nano-QSAR modeling in nanotoxicology and is expected to enhance the advancement of more secure nanomaterials in the future.

Key words: Neural network, computational, bioactivity, metal oxide nanoparticles, descriptors.

Introduction

Toxicity evaluation of nanoparticles isn't that straightforward on the grounds that each nanoparticle shows interesting morphological and physicochemical properties (1). The accessible and currently used testing frameworks may not be reasonable for screening nanoparticles because it is deficient in risk assessment protocols required for safety evaluation (2). Besides, scarcity of information on nanoparticle exposure is another restricting variable in nanotoxicological studies which hampers our conception with respect to nanoparticle toxicity mechanisms (3). Since nanoparticles may be metabolized or altered in vivo executing various consequences for the biosystems contrasted with their bulk counterparts (4). In this way, improved nanoparticle characterization methodologies are expected to eliminate uncertainties occurring at the level of exposure and hazard assessment to help risk executives in making regulatory policy decisions (5,6). Customary methodologies being utilized in nanoparticle risk assessment contrasted the novel nanoproducts and the regular non-nanoproducts bringing about raising vulnerabilities about the propriety of the current nanotoxicity related information (7).In addition, absence of a standard arrangement for archiving the probationary nanotoxicity data results in difficulty in data searching and gathering of obscure insights for building insilico models to address nano-explicit toxicity (8).To adapt to the current inadequacies in nanotoxicological studies, the first and the premier advance is to foster a normalized structure for detailing nanotoxicity data and also to lay out a completely organized datasets of nanotoxicity utilizing computational tools (9).Such insilico systems could be utilized to distinguish and reduce health and environmental hazards associated with individual nanoparticles. For this reason, it is expected to build databases of case narratives for the evaluation of nanoparticle related risks and for extra investigational studies on nanotoxicity resulting more than adequate data ought to be gathered with comprehensively acknowledged appraisal standards for information quality (10). Thus, this study aims at developing reliable computational models, thereby providing more insight on metal oxide nanoparticles cytotoxicity towards *Escherichia coli*

Materials and methods

For the development of nanoQSAR model, 17 metal oxide nanoparticles (ZnO, CuO, Y_2O_3, Bi_2O_3, In_2O_3, Al_2O_3, Fe_2O_3, SiO_2, SnO_2, V_2O_3, TiO_2**,** Sb_2O_3, ZrO_2, CoO, NiO, Cr_2O_3 and La_2O_3) for *Escherichia coli* were included in the study. The selection of the above-mentioned metal oxide nanoparticles depends on the availability of their associated toxicity data towards *Escherichia coli* (affecting viability). The obtained toxicity data for *Escherichia coli* is expressed in terms of logarithmic values of molar $1/EC_{50}$ (the effective concentration of a given metal oxide nanoparticle causing reduction of bacteria viability by 50%), which was considered as a dependant variable. Since nanopowder form of all metal oxide nanoparticles give rise to similarly sized aggregates in water suspension, regardless of their size (11). Therefore, the considered size range i.e., 15 to 90 nm for the selected metal oxide nanoparticles did not contribute to the cytotoxic mechanism towards *Escherichia coli.* Thus, the size effect of the selected metal oxide nanoparticles is nullified here. In the given study, logarithmic values of molar $1/EC_{50}$ were the observed toxic endpoint for *Escherichia coli* for which nanoQSAR modeling was done. Computation of three size-independent nanodescriptors for the selected metal oxide nanoparticles includes sum of metal electronegativity for individual metal oxide divided by the number of oxygen atoms present in a particular metal oxide ($\sum\chi$=nO) and metal cation charge corresponding to a given oxide (χ_{ox}). These nanodescriptors were easily obtained from their molecular formula and information acquired from the periodic table (12). However gaseous cation enthalpy of formation (ΔH_{Me+}) nanodescriptors were calculated using computational software PaDEL descriptor (v. 2.20) (13). Computed nanodescriptors were then used as a machine learning input in Weka platform employing machine learning algorithms including multiple linear regression (MLR) (14) and neural networks (NN) (15) for model development. In addition, different modules in Weka were used for model validation and models performance analysis. In the present study, validation was achieved by randomly splitting the dataset into training set including ZnO, CuO, Y_2O_3, Bi_2O_3, In_2O_3, Al_2O_3, Fe_2O_3, SiO_2, SnO_2 and TiO_2 and test set also called as validation set including V_2O_3, Sb_2O_3, ZrO_2, CoO, NiO, Cr_2O_3 and La_2O_3. Internal validation of the training sets was performed to measure the goodness

of fit and robustness of the developed nanoQSAR model using statistical parameters including Coefficient of determination between the experimental and predicted value i.e., least squares fit (R2), Root Mean Square Error (RMSE) etc (16, 17).In the current study the external validity of the developed nanoQSAR models were checked by the test set using externally validated determination coefficient Q^2_{Ext} and the root mean square error of prediction (RMSEP).Furthermore applicability domain of the developed nanoQSAR model was calculated for species training set employing leverage approach (18) for the analysis of model's prediction performance. Finally, interpretation of the developed nanoQSAR models to recognize the most suitable metal oxide nanoparticles associated toxicity mechanisms in *Escherichia coli.* In addition, comparison of the developed models for *Escherichia coli* on the basis of their F-measure was also performed.

Results

Based on the obtainable experimental data for the specific target endpoints i.e., $1/EC_{50}$ a multiple linear regression (MLR) and neural network (NN), based nanoQSAR models were developed to describe the relationship of the studied metal oxide nanoparticles versus the structural parameters involved in exhibiting cytotoxic effects in *Escherichia coli* , The observed cytotoxicity experimental values expressed in terms of logarithmic values of molar $1/EC_{50}$ for *Escherichia coli* is given in Table 1.

Table 1: Observed cytotoxicity values of 17 metal oxides nanoparticles for *Escherichia coli* (log $1/EC_{50}$)

S.no	Metal oxide NPs	Observed log $1/EC_{50}$ (mol l^{-1})
1	ZnO	3.45
2	CuO	3.20
3	V_2O_3	3.14
4	Y_2O_3	2.87
5	Bi_2O_3	2.82
6	In_2O_3	2.81
7	Sb_2O_3	2.64
8	Al_2O_3	2.49
9	Fe_2O_3	2.29
10	SiO_2	2.20
11	ZrO_2	2.15
12	SnO_2	2.01
13	TiO_2	1.74
14	CoO	3.51
15	NiO	3.45
16	Cr_2O_3	2.51
17	La_2O_3	2.87

Computed constitutional and quantum chemical nanodescriptors values for the studied metal oxides nanoparticles is given in Table 2.

Table 2: Computed values of nanodescriptors for 17 metal oxides nanoparticles

S.no	Metal oxide NPs	$\sum\chi$=nO	χ_{ox}	ΔH_{Me+} (Kcal mol^{-1})
1	ZnO	1.65	2	662.44
2	CuO	1.9	2	706.25
3	V_2O_3	1.087	3	1097.73
4	Y_2O_3	0.813	3	837.15
5	Bi_2O_3	1.347	3	1137.40
6	In_2O_3	1.187	3	1271.13
7	Sb_2O_3	1.367	3	1233.06

8	Al_2O_3	1.073	3	1187.83
9	Fe_2O_3	1.220	3	1408.29
10	SiO_2	0.950	4	1686.38
11	ZrO_2	0.665	4	1357.66
12	SnO_2	0.980	4	1717.32
13	TiO_2	0.770	4	1575.73
14	CoO	1.880	2	601.80
15	NiO	1.910	2	596.70
16	Cr_2O_3	1.107	3	1268.70
17	La_2O_3	0.733	3	1017.22

The predicted cytotoxicity values of $1/EC_{50}$ for the studied metal oxide nanoparticles is given in Table 3. Figure 1 and 2 displays the plot of experimentally observed versus predicted values of $1/EC_{50}$ obtained from the developed nanoQSAR models respectively.

Table 3: Predicted cytotoxicity values of 17 metal oxide nanoparticles for *Escherichia coli* (log $1/EC_{50}$) from the developed nanoQSAR models

S.no	**Metal oxide NPs**	**Observed $1/EC_{50}$ ($Mol\ l^{-1}$)**	**Predicted $1/EC_{50}$ ($Mol\ l^{-1}$)**	
			MLR	**NN**
1	ZnO	3.45	3.29	3.44
2	CuO	3.20	3.24	3.47
3	V_2O_3	3.14	2.74	2.79
4	Y_2O_3	2.87	3.07	2.99
5	Bi_2O_3	2.82	2.69	2.89
6	In_2O_3	2.81	2.52	2.55
7	Sb_2O_3	2.64	2.57	2.73
8	Al_2O_3	2.49	2.62	2.63
9	Fe_2O_3	2.29	2.34	2.43
10	SiO_2	2.20	1.99	2.07
11	ZrO_2	2.15	2.41	1.60
12	SnO_2	2.01	1.95	1.97
13	TiO_2	1.74	2.13	1.85
14	CoO	3.51	3.37	3.46
15	NiO	3.45	3.38	3.46
16	Cr_2O_3	2.51	2.52	2.53
17	La_2O_3	2.87	2.84	2.51

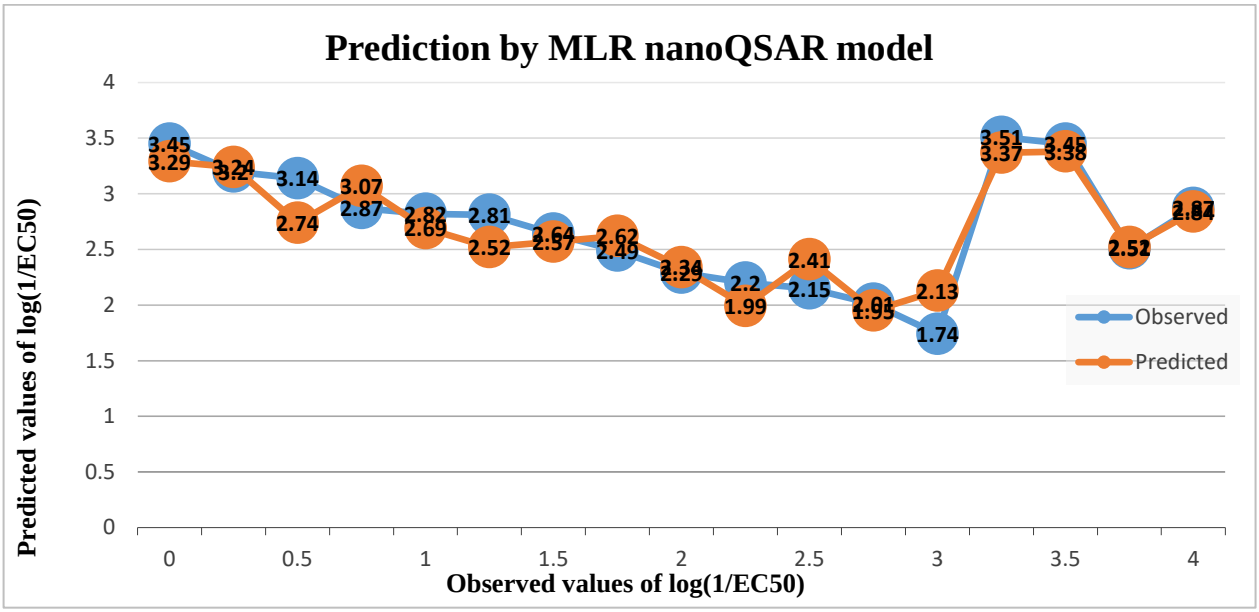

Figure 1: Plot of observed versus predicted log values of 1/EC$_{50}$ for MLR model

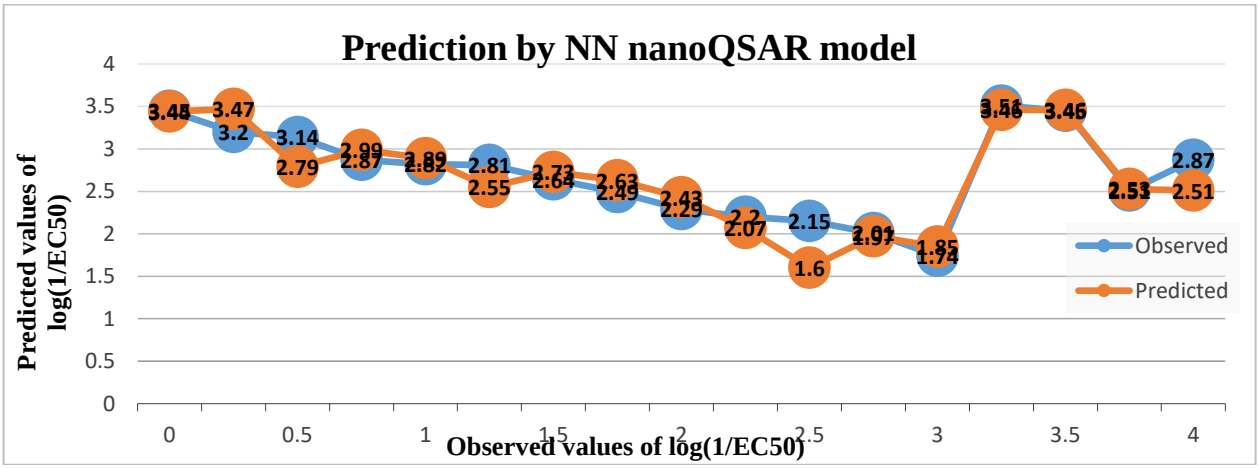

Figure 2: Plot of observed versus predicted log values of 1/EC$_{50}$ for NN model

Results of internal validation on the training sets for *Escherichia coli* is given in Table 4.

Table 4: Internal validation performed on the training sets for *Escherichia coli* on the developed nanoQSAR models

Escherichia coli									
S.no	**Model**	**Data set**	**No of NPs**	**No of descriptors**	**R^2**	**R^2_{adj}**	**Q^2_{cv}**	**RMSE**	**RMSECV**
1	MLR	Training	10	3	0.92	0.85	0.83	0.19	0.30
2	NN	Training	10	3	0.96	0.92	0.75	0.15	0.37

Results of external validation on test sets for both *Escherichia coli* is given in Table 5

Table 5: External validation performed on the test sets for both *Escherichia coli* on the developed nanoQSAR models

Escherichia coli						
S.no	**Model**	**Data set**	**No of NPs**	**No of descriptors**	**Q^2_{Ext}**	**RMSEP**
1	MLR	Test	7	3	0.93	0.19
2	NN	Test	7	3	0.93	0.28

The computed applicability domain critical leverage value for the training sets is given below:
h*(critical leverage value) = 1.5
The calculated leverage value for each metal oxide nanoparticle (h_i) is given in Table 6

Table 6: Computed leverage values of the studied metal oxides nanoparticles for *Escherichia coli*

S.no	Metal oxide NPs	*Escherichia coli*
1	ZnO	1.31
2	CuO	1.28
3	V_2O_3	1.01
4	Y_2O_3	1.21
5	Bi_2O_3	1.15
6	In_2O_3	1.08
7	Sb_2O_3	1.13
8	Al_2O_3	1.21
9	Fe_2O_3	1.14
10	SiO_2	1.25
11	ZrO_2	1.02
12	SnO_2	1.20
13	TiO_2	1.11
14	CoO	1.37
15	NiO	1.39
16	Cr_2O_3	1.12
17	La_2O_3	1.13

Evaluation results of the compared nanoQSAR models built using MLR and NN algorithms for *Escherichia coli* is given in Table 7.

Table 7: Evaluation results of the two nanoQSAR models

		Performance (F-measure at 0.05 level of significance)	
S.no	**Algorithms**	***Escherichia coli***	
1	**MLR**	0.85	
2	**NN**	0.74	

Discussion

From the data showed in Table 4 we can derive that the descriptors χ=nO, χ_{ox} and ΔH_{Me+} are critical at 83% certainty level for MLR and 75% certainty level for NN while anticipating the test set information. The models, MLR and NN could explicate 92% and 96% of the variance (R^2) respectively while it could anticipate 83% and 75% of the cross-validated predicted variance (Q^2_{cv}). Our internal validation results are comparable with the outcomes of Kar et al, (2016) (19) for MLR based model, and Fjodorova et al, (2017) (20) for NN. In any case, remembering this that the previous studies however employed similar algorithms as we did yet utilize different sort/number of nanodescriptors, different composition of metal oxide nanoparticles training/test sets as well as different model development insilico tools hence exact critical evaluation of the statistical quality of our built models with the previously reported ones is not always possible. The anticipated toxicity assessments of 17 metal oxide nanoparticles against *Escherichia coli* is given in Table 3. Figure 1 and 2 displays the distribution of the two nanoQSAR models determined predicted values which were seen to be adjusted exceptionally near their corresponding experimental values. Consequently, for the studied metal oxides nanoparticles, a good agreement between the observed and those anticipated by the nanoQSAR models were noticed (21). In the ongoing study, our developed models uncovered that the descriptor χ_{ox} has a negative coefficient towards toxicity i.e., Cytotoxicity of metal oxide

nanoparticles diminishes with an increase in their cationic charge (22). On the other hand, our models also revealed that an increased cationic charge (χ_{ox}) results in a positive value increase of ΔH_{Me+} and in return decreased cytotoxicity log(1/EC_{50}) is observed. Besides, both of our developed models have good internal stability and statistical robustness signifying that the models were not obtained by chance as verified by internal and external validation metrices (23). On the other hand, obtained external validation parameters i.e., Q^2_{Ext} and RMSEP (Table 5) revealed confirmation that our developed models are applicable for predicting toxicity of any other metal oxide nanoparticles if their structures fall within the applicability domain of the training set $h_i < 1.5$ for *Escherichia coli*. In addition, Comparison analysis of the developed models based on F-measure metrices revealed that the value of F-measure for linear MLR based model was higher i.e., 85% than non-linear NN model i.e.,74% indicating improved prediction competence of linear MLR model over non-linear NN model in *Escherichia coli*. Thus, our developed nanoQSAR models for *Escherichia coli* were consistent since it has passed all the internal/external validation metrices and have satisfied all the criteria's required for its statistical acceptance.

Conclusion

Thus, MLR based QSAR model displayed high statistical robustness in the proposed specie for toxicity profiling.

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