WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 14, 411-423. Research Article ISSN 2277–7105

PREDICTIVE TOXICITY ASSESSMENT OF TOXINS RELEASED FROM *MICROCYSTIS AERUGINOSA* KUTZ: AN *IN SILICO* APPROACH

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Article Received on
05 Sept. 2017,

Revised on 25 Sept. 2017,
Accepted on 15 October 2017

DOI: 10.20959/wjpr201714-9887

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ABSTRACT

Microcystis aeruginosa Kutz commonly occurring toxin producing cyanoprokaryote of tropical and temperate countries found in aquatic bodies. Present study deals with the prediction of ecotoxicity of toxins of *Mycrocystis* on algae, daphnid and fish and also mutagenicity, carcinogenicity, through an *in silico* approach with special reference to QSAR modelling and toxicokinetics (ADMET) study. In predictive results, out of six compounds three compounds viz. anatoxin-a, microcystin-LR and LA were observed to be toxic to three test models and the ADMET study revealed that anatoxin-a was neurotoxin having positive capacity to penetrate the blood brain barrier and P-glycoprotein inhibitor. All compounds were non-inhibitor except two compounds viz. microcystin-LR and LA and for metabolism, all the compounds were observed non-inhibitor for CYP450 2C9, 2D6 and 3A4 enzymes inhibitor while for CYP450 2C9, 2D6 and 3A4 enzymes substrate, microcystin-LA was found substrate inhibitor and anatoxin-a, microcystin-LR and RR were substrate and saxitoxin and neosaxitoxin were found non-substrate for CYP450 3A4 but all the compounds were showed non-substrate for CYP450 2C9 and 2D6 enzyme substrate but all compound were observed to be non-mutagenic and non-carcinogenic. In conclusion, the bioactive compounds found in *M. aeruginosa*, few of these were showed toxic to algae, daphnid and fish when studied through QSAR modelling tool as ECOSAR. This predictive screening can be suitable to detect ecotoxicological risk assessment. It is suggesting to perform future experimental study to validate the present predictive results.

KEYWORDS: *M. aeruginosa*; Phytotoxins; QSAR modelling, Toxicokinetics, Ecotoxicity prediction.

INTRODUCTION

M. aeruginosa belonging to cyanoprokaryote, member of cyanophyceae produces toxin, which is basically different combinations of a number of peptide or peptide-containing toxins of undefined structure.[1] The hepatotoxin known as microcystin was first isolated from *Microcystis* sp.[2,3] The toxins are mainly microcystins (MC), obtained as secondary metabolites.[4,6] In many cases, the surface water used to become threat due to the presence of *M. aeruginosa* that lead to toxicity for domestic and wild animals, even for human beings.[7,13] It has been established that *M. aeruginosa* produces hepatotoxin called as microcystin while anatoxin-a, saxitoxin and neosaxitoxin are neurotoxin, derived as neurotoxic alkaloids.[14,16] According to Duy et al.,[17] the toxin of different microcystin is named on the basis of amino acid composition due to methylation or demethylation at selected sites within the cyclic peptide.[14] The microcystin congeners are microcystin-LR, microcystin-RR, microcystin-LA, etc.[18,19]

Several studies have been reported by using the toxins released from *M. aeruginosa* in vitro on cell lines such as liver (Mahlavu and PLC/PRF/5), lung (MRC-5), cervix (HeLa), ovary (CHO-Kl) and kidney (BGM, MA-104 and Vero) cell lines[1,20] and in vivo experimental assay in different organ systems/tissues of animals viz. liver, kidney, digestive tract, gonads, immune system, hypothalamic-pituitary system, and nervous system.[1,21,26] It has already been established that toxins of microcystin are potent mutagenic, carcinogenic and tumorigenic in the experimental assay. [1,26,27] On the other hand, these toxins have potential toxic effect to lower animals such as protozoans, daphnids, fishes, birds, etc.[1,3,6,27,32]

An *in silico* study with special reference to quantitative structure-activity relationship (QSAR), is an important predictive mathematical model, which has a relationship between the biological activity and the two-dimensional or three-dimensional compounds descriptors.[33,36] These supported several ecotoxicological endpoints for aquatic animals.[6,37,39] Lipnick[40] stated that QSAR modelling very important tool for testing of untested chemicals to meet regulatory requirements at priority level. Another tool is toxicokinetics, in which ADMET (absorption, distribution, metabolism, excretion and toxicity) properties can easily be known for each test compound.[41,45]

It was an attempt to predict toxicity, mutagenicity, carcinogenicity and ADMET for established toxins of *Microcystis aeruginosa* Kutz through an *in silico* approach with special reference to QSAR modelling and toxicokinetics study.

MATERIALS AND METHODS

Selection of compounds from *M. aeruginosa*

The established toxins from *M. aeruginosa* were selected from the literatures.[16,19] The selected phytocompounds are anatoxin-a, saxitoxin, neosaxitoxin, microcystin-LR, microcystin-LA and microcystin-RR (Fig 1).

QSAR modelling for predictive toxicity evaluation

The Quantitative Structure Activity Relationship (QSAR) modelling was done by using ECOSAR (Ecological Structure Activity Relationship) tool (Version, 1.11) developed by (Mayo-Bean et al.[46] In the present study, the parameters selected were EC_{50} value (mg/L) for green algae, LC_{50} value (mg/L) of daphnid and fish against selected toxins released from *M. aeruginosa*.

ADMET prediction

ADMET-SAR (absorption, distribution, metabolism, excretion, toxicity – structure activity relationship) was used to check whether the compound has fulfilled the conditions as drug candidate.[42]

RESULTS AND DISCUSSION

The present predictive toxicity results indicated for toxins such as anatoxin-a, saxitoxin, neosaxitoxin, microcystin-LR, microcystin-LA and microcystin-RR released from *M. aeruginosa* through QSAR modelling and toxicokinetics study by using ECOSAR and ADMET-SAR tool.

The present predictive baseline acute toxicity (EC_{50}) and (LC_{50}) results (mg/L) were indicated that high toxicity values were obtained for anatoxin-a, followed by microcystin-LA and microcystin-LR in green algae as also observed in daphnid and fish. Less toxic effect was observed for saxitoxin, followed by neosaxitoxin and microcystin-RR in all test organisms (Table 1).

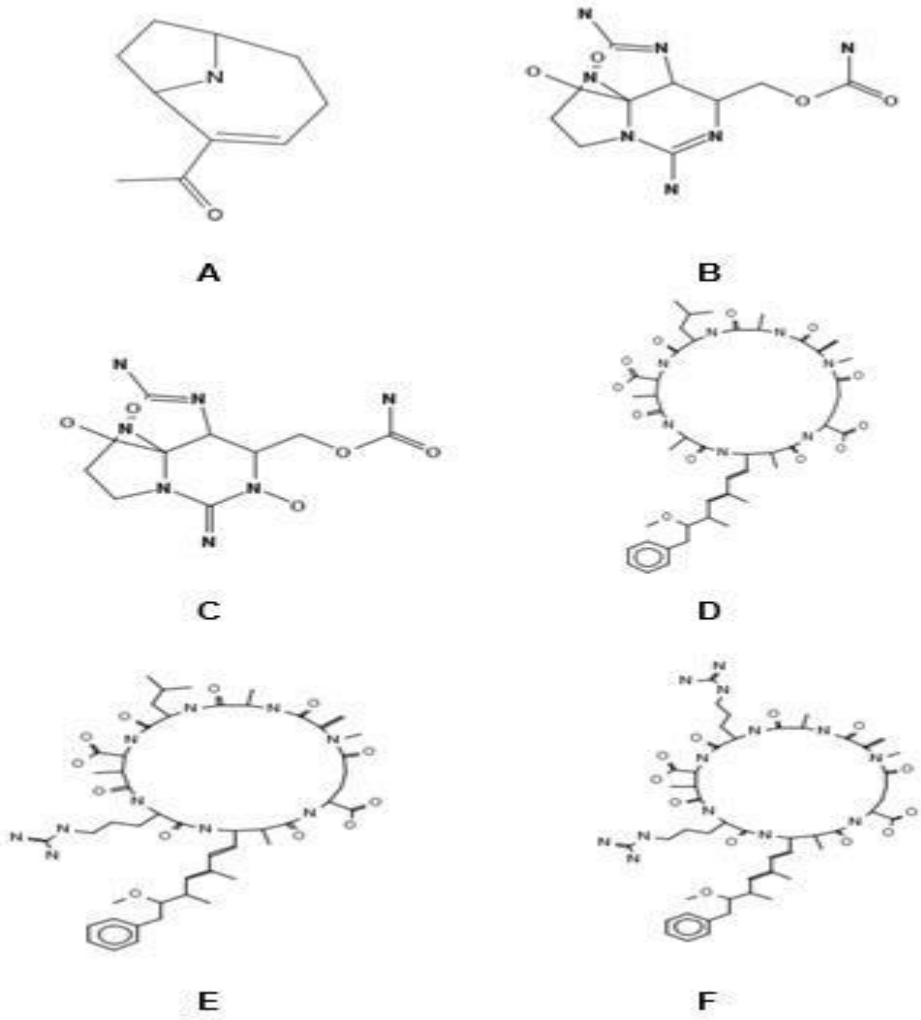

Fig. 1. Different compounds selected from *Microcystis aeruginosa* (A = Anatoxin-a; B = Saxitoxin; C = Neosaxitoxin; D = Microcystin–LR; E = Microcystin–LA and F = Microcystin–RR).

Table 1. Predictive baseline toxicity data on different species exposed to toxic compounds released from *Microcystis aeruginosa.*

Compounds	CAS No.*	Green algae 96h EC_{50} (mg/L)	Daphnid 48h LC_{50} (mg/L)	Fish 96h LC_{50} (mg/L)
Anatoxin-a	64285-06-9	232.53	436.40	833.45
Saxitoxin	35523-89-8	2100000	30400000	95000000
Neosaxitoxin	64296-20-4	984000	11700000	34900000
Microcystin–LR	101043-37-2	2738.37	6034.62	11981.52
Microcystin–LA	96180-79-9	566.67	874.80	1593.63
Microcystin–RR	111755-37-4	118000	631000	1550000

*taken from PubChem database.

The microcystin toxin, microcystin-LR has been prescribed the standard limit in drinking water for human consumption at a concentration of 1µg\L[47] but researchers have

documented that 0.01 µg/L led to primary liver cancer from ditches, ponds, river water of certain part of China.[27,48] In other studies, *Daphnia* sp. showed toxic effect when exposed to microcystins in aquatic systems[49,51] while different species of fishes have showed toxicity by microcystins even in lower value of µg\L.[52] In this context, it was reported in experiment that dose exposed through i.p. injection for microcystin-LR (20-1500µg\L) proved much toxicity in fish rather than other route of exposure like gavage or oral feeding.[53] Interestingly, researchers have found in their experiment that microcystin toxin at a concentration of 50µg/kg exposed through i.p. injection in the carp (*Cyprinus carpio*) showed mortality while gavage of 250 µg/kg in similar carp showed no mortality.[54] According to Liu et al.,[55] developing embryos are more susceptible than juveniles of loach (*Misguruns mizolepis* Gunthe) to microcystin-LR when exposed as solutions for chronic studies. Their experiment revealed that LC_{50} value were 164.3µg/L for embryos and 593.3µg/L for small hatched juveniles.

Regarding QSAR modelling for determination of LD_{50} values for 24 types of microcystins have been obtained in the observed versus predictive values through determined molecular descriptors with statistical interpretation,[56] but in the present study, EC_{50} and LC_{50} values (mg/L) in algae, daphnid and fish by using QSAR modelling software has been found to be better understand the toxicity values of different compounds generated from *M. aeruginosa* prior to tedious, expensive and costly laboratory experiments. The researches have been found in each test species such as daphnid and fish, but works are lacking on toxicity on algae other than cyanobacteria by the exposure of different compounds of *M. aeruginosa*. It has already been established by US regulatory guideline that algae, daphnid and fish should be used for experimental and/or predictive measurement in acute toxicity study of chemical compounds.[57] For predictive toxicity analysis ECOSAR tool is more suitable for ecotoxicological predictive assessment for compounds, where experimental data are lacking.[57]

In Table 2, the results showed ADMET prediction for six bioactive compounds. The absorption parameters such as BBB (blood brain barrier), HIA (human intestinal absorption) and Caco-2 (carcinoma cell permeability), P-glycoprotein inhibitor and P-glycoprotein substrate; the distribution parameter is based on organelle location; the metabolism parameters viz. CYP450 inhibitor and substrate (2C9, 2D6 and 3A4 enzymes); the excretion parameter is ROCT (renal organic cation transporter) and toxicity parameters such as

gradation for acute oral toxicity, high or low levels in fish and honeybee toxicity as well as mutagenicity and carcinogenicity data for these compounds. The predictive results indicated that only anatoxin-a is BBB, HIA and Caco-2 positive and rest of the compounds were negative for these parameters. For P-glycoprotein inhibitor, all compounds showed non-inhibitor except two compounds viz. microcystin-LR and LA while for P-glycoprotein substrate, three compounds such as anatoxin-a, saxitoxin and neosaxitoxin showed substrate non-inhibitor but rest three compounds viz. microcystin-LR, LA and RR were found to be substrate inhibitor. In the distribution as subcellular localization, first three compounds located in mitochondria while rest three compounds located lysosome of affected organisms. All the compounds were found non-inhibitor for renal organic cation transporter.

In case of metabolism parameter, all the compounds were observed non-inhibitor for CYP450 2C9, 2D6 and 3A4 enzymes inhibitor while for CYP450 2C9, 2D6 and 3A4 enzymes substrate, microcystin-LA was found substrate inhibitor and anatoxin-a, microcystin-LR and RR were showed as substrate specific and saxitoxin and neosaxitoxin were found non-substrate specific for CYP450 3A4 but all the compounds were showed non-substrate for CYP450 2C9 and 2D6 enzyme substrate. In toxicity screening, grade I toxin, three compounds such as saxitoxin, microcystin-LR and RR were observed and rest three compound were found as grade III toxin. In case of toxicity of fish, low level was found as saxitoxin and neosaxitoxin and rest all four observed in high level. In case of honeybee toxicity, anatoxin-a and microcystin-LR showed in high level while rest four compounds observed in low level. In this present prediction, all the compounds were found to be non-mutagenic and non-carcinogenic. The BBB penetration results revealed that anatoxin-a was a neurotoxin due to BBB positive but rest compounds were BBB negative. In general, BBB penetration leads to damage of central nervous system.[44,45,58] According to Shadrack and Ndesendo,[58] the parameters for metabolism prediction CYP450 2D6 and 3A4 inhibitor enzymes are suitable for identification of drugs or toxins. The researchers have emphasized for compounds that CYP450 enzymes help in phase I metabolism.[58,59] The present ADMET study is beneficial to know ecotoxicological risk assessment for the bioactive compounds released from *M. aeruginosa* in aquatic bodies.[44]

Table 2. ADMET-Prediction profiles for phytochemicals of *M. aeruginsa.*

Absorption							**Distribution**
Sl. No.	**Phytoligands**	**Blood-brain barrier**	**Caco-2 permeability**	**Human intestinal absorption**	**P-glycoprotein inhibitor**	**P- glycoprotein substrate**	**Subcellular localization**
1.	Anatoxin-a	BBB+	Caco2+	HIA+	NI	SNI	Lysosome
2.	Saxitoxin	BBB-	Caco2-	HIA-	NI	SNI	Lysosome
3.	Neosaxitoxin	BBB-	Caco2-	HIA-	NI	SNI	Lysosome
4.	Microcystin–LR	BBB-	Caco2-	HIA-	I	SI	Mitochondria
5.	microcystin–LA	BBB-	Caco2-	HIA-	I	SI	Mitochondria
6.	Microcystin–RR	BBB-	Caco2-	HIA-	NI	SI	Mitochondria

Metabolism								**Excretion**
Sl. No.	**Phytoligands**	**CYP450 2C9 inhibitor**	**CYP450 2C9 substrate**	**CYP450 2D6 inhibitor**	**CYP450 2D6 substrate**	**CYP450 3A4 inhibitor**	**CYP450 3A4 substrate**	**ROCT**
1.	Anatoxin-a	NI	NS	NI	NS	NI	S	NI
2.	Saxitoxin	NI	NS	NI	NS	NI	NS	NI
3.	Neosaxitoxin	NI	NS	NI	NS	NI	NS	NI
4.	Microcystin–LR	NI	NS	NI	NS	NI	S	NI
5.	microcystin–LA	NI	NS	NI	NS	NI	SI	NI
6.	Microcystin–RR	NI	NS	NI	NS	NI	S	NI

Toxicity						
Sl. No.	**Phytoligands**	**Acute oral toxicity**	**Fish toxicity**	**Honey bee toxicity**	**AMES toxicity**	**Carcinogens**
1.	Anatoxin-a	III	High FHMT	High HBT	NT	NC
2.	Saxitoxin	I	Low FHMT	Low HBT	NT	NC
3.	Neosaxitoxin	III	Low FHMT	Low HBT	NT	NC
4.	Microcystin–LR	I	High FHMT	High HBT	NT	NC
5.	microcystin–LA	III	High FHMT	Low HBT	NT	NC
6.	Microcystin–RR	I	High FHMT	Low HBT	NT	NC

NI = Non-inhibitor; I = Inhibitor; NS = Non-substrate; NSI = Non-substrate inhibitor; S = Substrate; SI = Substrate inhibitor; ROCT = Renal Organic Cation Transporter; I = Category I (LD_{50} values less than or equal to 50mg/kg); II = Category II (LD_{50} values greater than 50mg/kg but less than 500mg/kg); III = Category III (LD_{50} values greater than 500mg/kg but less than 5000mg/kg) and IV = Category IV (LD_{50} values greater than 5000mg/kg); H = High; L = Low; FHMT = Fathead minnow toxicity; HBT = Honey bee toxicity; NT = Non-toxic; NC = Non-carcinogen

CONCLUSION

It is concluded from the present predictive results that the bioactive compounds found in *M. aeruginosa*, few of these showed toxicity to algae, daphnid and fish in the aquatic ecosystem, known through QSAR modelling software by using ECOSAR tool. In the present study, the predictive toxicity values (EC_{50} and LC_{50}) for all compounds in three test models observed >200mg/L, which means individual bioactive compound is showing least toxicity but it may be more toxic as cumulative effects to the present test models and/or toxicity at chronic level may develop due to the failure of metabolic activities by inhibiting CYP450 in higher animals. Presently, ADMET prediction revealed that all compounds were non-mutagenic and non-carcinogenic after screening by using ADMET-SAR tool. On the other hand, ADMET study also observed that all compounds obtained negative results for penetration BBB and HIA and Caco-2 permeability except anatoxin-a. For the metabolism parameter, it was observed that all compounds are non-inhibitor and non-substrate specificity for CYP450 2C9, 2D6 and 3A4, but for CYP450 3A4 substrate activity, three compounds namely anatoxin-a, microcystin-LR and RR were substrate specific, two compounds viz. saxitoxin and neosaxitoxin were non-substrate specific and microcystin-LA was substrate inhibitor. In context with the present predictive results, it is suggesting to carry out experimental study to validate the present *in silico* results for each compound as well as combination of compounds to know experimental eco-toxicity of compounds generated in *M. aeruginosa*.

ACKNOWLEDGEMENT

Authors are thankful to the developers of both software and database of PubChem for present bioactive compounds used in the present study and Centre of Advanced Study, Department of Botany, Calcutta University, Kolkata, India for infrastructural facilities.

CONFLICT OF INTEREST

No conflicts of interest for the present study.

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