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On the Potential of Phytochemical Remedy for Envenomation and the Consequent Endocrinopathy, with a Note on Conservation – A Case Study of Venom Informatics

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Abstract

Envenomation is a serious neglected health issue at the global level that affects millions of people every year. It is highly prevalent among farmers and rural natives and is mainly due to the bite from snakes, spiders, frogs, dogs, wasps, bees, ants, etc. Many plants with antidote potential grow around our backyard without proper recognition and, unfortunately, several of them are under threat of extinction due to human interference and other environmental factors. The sustainable utilization of those antidote herbs can benefit as a life saver to the needy patients. The herbs can also be farmed and used commercially for the pharmaceutical application and incorporated with biotechnology and bioinformatics with a vision of synthesizing antidote drugs with less or no side effects. The objective of the present work is to create awareness among the public for the wise use of wild and local herbs, and their sustainable utilization with a computational case study on laboratory experiments done in two traditional plant based antidotes, selected from literature. The investigation is focused on *Daboia russelii* venom neutralization via *in silico* approach which can significantly reduce the time, expense, labour and samples taken. Our approach will add to the conventional non-specific polyvalent anti-snake venom (ASV) with more specific plant-based antidotes.

Keywords: Antidote, Envenomation, Herbal Medicines, Sustainable Utilization, Venom Informatics

1. Introduction

The term "envenomation" describes the process by which venom is injected into animals and or men by the bite or sting of a venomous species [1]. Millions of envenomation occurs every year. Precise statistical data in this regard are not available since majority of the morbidity/mortality rate is under-reported by either the hospitals or the local healers or the reported data may be fragmentary[2]. Among the animal bites, the most important are from snakes and up to five million people are affected at the global level by snake bites alone. An estimated approximately 2.4

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million people are bitten by venomous snakes and 94,000-125,000 deaths per year are annually reported with an additional 4 00,000 amputations and other severe health consequences [3]. South Asia is one of the regions where the highest burden of mortality and morbidity due to snake bites occur, especially in the Indian subcontinent. More than 200,000 snake bite cases, with more than 35,000-50,000 deaths, are reported in India [3, 4, 5]. Most of these venomous bites that lead to human lethality are due mainly to the "Big Four" venomous snakes, *viz*. *Naja naja* (Spectacled cobra), *Bungarus caeruleus* (Common krait), *Daboia russelii* (Russell's viper) and *Echis carinatus* (Saw scaled viper). In Kerala, India, most snake bite deaths are due to *Daboia russelii* as it delivers the highest dose of venom to the victims in a single bite.

Among the many manifestations of envenomation is endocrinopathy. Case studies, with special reference to bite of Russell's viper, have reported severe hypofunction of pituitary gland accompanied by problems in secretion of insulin and thyroxine[6, 7, 8]. An experimental study in rat model, using venoms of four species of snakes from Saudi Arabia, revealed that testosterone levels were either normal or stimulatory with acute treatment or inhibitory with chronic treatment. Cortisol levels were normal at acute treatment but showed a gradual rise reflecting the stress imposed on the animals. The effects on insulin and thyroxine were similar to those of testosterone level showing normal or stimulatory effect with acute treatment followed by decreased levels of hormones with chronic treatment[9].

The severity will be in high risk if the victim is far from medical facilities, as well as the allergic reactions due to the modern polyvalent anti-sera treatments. The insufficient facility to store antivenoms and the lack of timely treatments increase the risk. The current treatment modalities such as polyvalent anti-snake venom (ASV) and antibiotics are very expensive and also produce adverse effects.

Nature has been a healing agent which has been recognized for centuries. The Western Ghats, one of the "hottest hot-spots" of Biodiversity[10, 11, 12], passing through the entire stretch of Kerala, and continued on the North as well as South, is enriched with a plethora of fauna and flora having diverse therapeutic properties. Traditional knowledge and scripts have used this rich source of medicinal plants for treating several curable and incurable diseases. Local traditional healers, especially the tribal communities, have amazing practical expertise in the traditional primary healthcare. Now-a-days, scientists are focusing on traditional ethnomedicinal and alternative therapy-based treatments since these are free from side-effects and cost-effective compared to the allopathic treatments including polyvalent antivenoms and antibiotics. Many plants with antidote properties found around our surroundings, including gardens and back-yards, are potent drugs for many insect and animal bites. The sustainable and proper utilization of these medicinal herbs can be primary aids for venomous bites and thus as a life saver to the victims. The current global changes and the human activities have made the valuable plants getting vanished. In addition to this, the increasing research interests in the analysis and evaluation study of phytochemicals are having a critical role in the significant reduction of availability of medicinal plants. The need for maintaining the resources of *Mother Nature* has to be taken into consideration for the well-being of life. The modern scientists are curiously investigating the scope of bringing the potential of medicinal herbs into a single drug-like capsule for its easy availability and administration.

Venom Informatics, a systematic Bioinformatics approach is one in which the venom data are stored in electronic repositories which can be used to analyse, interpret and predict the molecular level drug-toxin interaction using various advanced Computational Biology and Bioinformatics tools and software. With the aid of those Bioinformatics databases and software, the primary analysis, ADME (Absorption, Distribution, Metabolism and Excretion) and evaluation study for a particular biological activity at molecular level can be predicted for *in vivo*/*in vitro* experiments with the goal of reducing the huge collection of plant resources at primary level of analysis, time, expense and labour efforts taken to identify the best leads. The present work is based on such practices with a case study on two plant based antidotes, *Hemidesmus indicus* and *Strychnos nux vomica* identified from a review article [13]. The experimentally isolated and characterized metabolites such as 2-hydroxy-4-methoxybenzoic acid from the root of *Hemidesmus*

indicus and Caffeic acid from the seed of *Strychnos nux vomica,* which have undergone i*n vitro* and *in vivo* studies in experimental animals[14, 15] were used in our *in silico* molecular docking analysis lab to confirm the predictability of the computational software.

2. Materials and Methods

The selected secondary metabolites from the specified plants, *Hemidesmus indicus* and *Strychnos nux vomica,* were subjected to molecular docking studies against the potential target venom protein, Phospholipase A_2 (PLA_2) of Russell's viper. The molecular docking was performed using the commercial molecular software, Discovery Studio 4.0 (BIOVIA, USA) on the Microsoft Windows 8 operating system. It is a suite of software for simulating small molecule and macromolecule systems[16]. The ligand molecules were subjected to pre-docking steps such as molecular preparation and energy minimization to optimize the molecular stability using the software. The analysis of protein-ligand interaction, and thus the biological activity, was also computed using the simulation software.

2.1 Ligand Identification and Optimization

The antidote property showing secondary metabolites, 2-hydroxy-4-methoxybenzoic acid obtained from *Hemidesmus indicus* and Caffeic acid from *Strychnos nux vomica* were selected based on the perusal of literature[13] in which the extracted metabolites have shown significant molecular effect in neutralizing the toxicity of Russell's viper[14, 15]. The three-dimensional structures of the small molecules from the respective plants were selected from the open access chemical database, PubChem with the compound identifiers CID: 75231 and 689043, respectively[17, 18]. The downloaded ligand molecules were prepared and optimized using the protocols, Ligand Preparation and Ligand Minimisation in the Discovery Studio 4.0. It generates possible orientation of ligands as conformers. All the generated isomers of the ligand molecules were subjected to docking process to predict the best lead isomer from the receptor-ligand complexes.

2.2 Target Identification, Validation and Optimization

The 3D structure of the target protein was searched based on the group II snake venom secretory PLA_2 of *Daboia russelii* as it is well-studied among snake venom proteins. From the list of X-ray crystallographic structures classified under Hydrolase enzyme, the protein with PDB ID 1TH6 was retrieved from the Protein Data Bank. The protein was selected based on the good resolution at 1.23 A^o and Ramachandran plot. The protein consists of a single chain with 121 amino acid residues featured with 71 helices, 8 sheets and 42 loops/coils. The active site1 with functional residues Histidine 48, Aspartic acid 49 and Lysine 69 have been defined as binding sites. Prior to docking, the validated target protein was allowed to protein purification, preparation and minimization steps to globally optimize the protein structure to attain the stable confirmation using the Discovery Studio software.

2.3. Computational Molecular Docking

Molecular docking studies were conducted to evaluate the binding affinity of the selected ligand molecules to the target protein. The docking procedure was carried out using the force field, CHARMm-based CDocker algorithm. It is a grid-based molecular docking refinement method that generates maximum random conformations of protein-ligand complexes through High Temperature Molecular Dynamics. The docking parameters were kept as default. The docked poses listed as Hits were ranked according to the least CDocker energy which is calculated on the basis of internal ligand strain energy (-ve CDocker energy) and receptor-ligand interaction energy (-ve CDocker Interaction energy). The interaction energies calculated the non-bonded interactions such as Vander Waal's and electrostatic energies between two sets of atoms. Here, the highest value with –ve CDocker energy and –ve CDocker Interaction energy were considered as the favourable scores having least energies. All docked poses were further subjected to calculate the Binding Energy protocol to predict the best hit with least binding energy. The binding energy implies the stability of the receptor-ligand binding affinities. The filtered pose with least energies were further screened on the basis of number

of hydrogen bonds which implies the stability of the molecular interaction. The filtered best poses were then subjected to ADME, Mutagenicity and Carcinogenicity tests to predict the best non-toxic lead molecule.

3. Results and Discussion

It was observed that the least energy scores of -ve CDocker energy and -ve CDocker Interaction energy with 58.3086 kCal/mol and 64.3222 kCal/mol, respectively, have shown the least binding energy value (564.483 kCal/mol) among the hits obtained from the 1TH6 and 2-hydroxy-4-methoxybenzoic acid docked complexes (Table 1). The hydrogen bond interactions (Figure 1) with residues Lysine 69 with a bond distance of 2.00515 A° show the ligand as a significant antagonist of viper venom protein PLA_2. The other interaction residues are Glycine 32 with 5.54918 A° and Tryptophan 31 with 5.54918 A°. Similarly, the Caffeic acid has shown the best pose with the least binding energy (-309.016 kCal/mol) where -ve CDocker and -ve CDocker Interaction energies are 45.0078 kCal/mol and 47.0702 kCal/mol, respectively, with PLA_2 (Table 2). The interaction residues (Figure 2), Lysine 69 with a hydrogen bond distance of 1.96024 A° and Glycine 33 with 1.90193 A° also show antagonistic/inhibitory property to reduce the neurotoxic venom. It is also noted that Lysine 69 is the key residue that is having a significant role in inhibiting the venom PLA_2 activity. The ADME, Carcinogenicity and Mutagenicity tests predicted (Table 3) that 2-hydroxy-4-methoxybenzoic acid is less toxic with

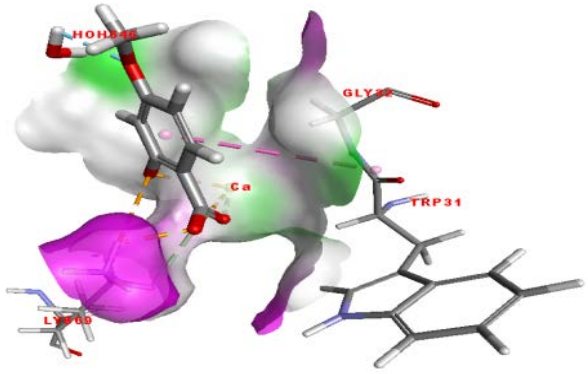

Figure 1. Selected docked pose and H-bond interactions of 2-hyroxy-4-methoxybenzoic acid with Russell's viper venom phospholipase A_2 in Discovery Studio software.

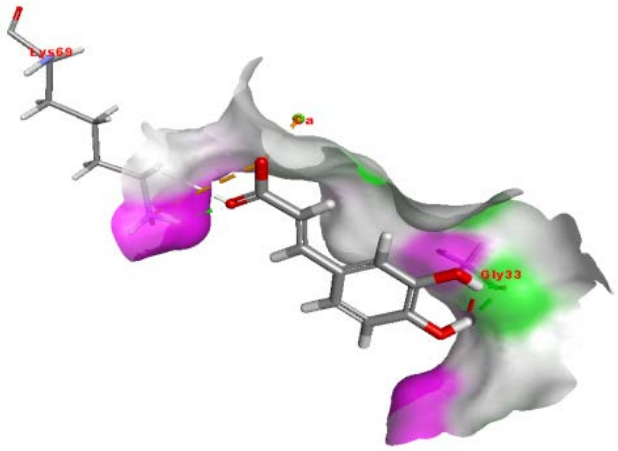

Figure 2. Selected docked pose and H-bond interactions of Caffeic acid with Russell's viper venom phospholipase A_2 in Discovery Studio software.

Table 1. Details of selected Hit molecule from *Hemidesmus indicus* against PLA_2 in Discovery Studio 4.0

Ligand	-CDocker Energy (Kcal/mol)	-CDocker IE (Kcal/mol)	BE (Kcal/mol)	Residues	H-bond Distance (A0)	No. of H-bonds	Potential Energy (Kcal/mol)	Van der waal's Energy (Kcal/mol)	Electrostatic Energy (Kcal/mol)
2-hydroxy-4-methoxy benzoic acid	58.3086	64.3222	-564.483	Lys69 Gly32 Trp31	2.00515 5.54918 5.54918	7	132.04488	2.06174	124.01204

Table 2. Details of selected Hit molecule from *Strychnos nux vomica* against PLA_2 in Discovery Studio 4.0

Ligand	-CDocker Energy (Kcal/mol)	-CDocker IE (Kcal/mol)	BE (Kcal/mol)	Residues	H-bond Distance (A0)	No. of H-bonds	Potential Energy (Kcal/mol)	Van der waal's Energy (Kcal/mol)	Electrostatic Energy (Kcal/mol)
Caffeic acid	45.0078	47.0702	-309.016	Lys69 Gly33	1.96924 1.90193	0	-21.50396	1.3162	-25.50056

Table 3. ADME, Mutagenicity and Carcinogenicity predictions of the selected ligand molecule from Discovery Studio 4.0

Ligands	Aqueous Solubility	BBB	Absorption Level	AlogP	Mutagenicity	Carcinogenicity
2-hydroxy-4-methoxy benzoic acid	4	3	0	1.201	0.000	0.000
Caffeic acid	4	3	0	1.443	0.964	0.027

no carcinogenic and mutagenic property when compared to Caffeic acid. Based on the interaction study and ADME prediction, 2-hydroxy-4-methoxybenzoic acid shows a significant neutralization effect when compared to Caffeic acid against Russell's viper venom phospholipase A_2.

4. Conclusions

The *in silico* approach used in the investigation has been validated and proves that the docking results are consistent with the laboratory experiments in animal models. Hence, the approach would strengthen the use of computational approach and analysis for an effective screening strategy and thereby conserve the potential plants for generations.

5. References

1. *https://en.m.wikipedia.org/wiki/Envenomation*
2. Chippaux JP. Snake-bites: appraisal of the global situation. Bull World Health Organ. 1998; 76(5): 515–52.
3. *http://www.who.int/mediacentre/factsheets/fs337/en/*
4. Bawaskar HS. Snake venoms and antivenoms: Critical supply issues. J Assoc Physicians India. 2004; 52: 11–13.
5. Kasturiratne A, Wickremasinghe AR, de Silva N, et al. The global burden of snakebite: A literature analysis and modelling based on regional estimates of envenoming and deaths. PLoS Medicine 2008; 5(11): e218.
6. Antonypillai CN, Wass JA, Warrell DA, Rajaratnam HN. Hypopituitarism following envenoming by Russell's vipers (*Daboia siamensis and D. russelii*) resembling Sheehan's syndrome: First case report from Sri Lanka, a review of the literature and recommendations for endocrine management. QJM 2011; 104: 97–108.
7. Tun-Pe, Phillips RE, Warrell DA, Moore RA, Tin-Nu-Swe, Myint-Lwin, et al. Acute and chronic pituitary failure resembling Sheehan's syndrome following bites by Russell's viper in Burma. Lancet 1987; 2: 763–767.
8. Saxena A, Srivastava AK, Rajput AS, Tiewsoh I, Jaioo UN. Acute hypopituitarism – a rare complication of vasculotoxic snake bite: A case report. J Mahatma Gandhi Inst Med Sci, 2014; 19: 144–147.
9. Abdel-Galil KA, Al-Hazimi AM. Effects of snake venom from Saudi cobras and vipers on hormonal levels in peripheral blood. Saudi Med J, 2004, 25(8):1080–1085.
10. *http://whc.unesco.org/en/list/1342*

11. Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GA, Kent J. *Biodiversity hotspots for conservation priorities. Nature 2000; 403: 853–858.*
12. *https://timesofindia.indiatimes.com/india/UN-designates-Western-Ghats-as-world-heritage-site/articleshow/14610277.cms*
13. Gupta YK, Peshin SS. Snake bite in India: Current scenario of an old problem. J Clin Toxicol. 2014; 4:182. doi: 10.4172/2161-0495.1000182.
14. Alam MI, Auddy B, Gomes A. Isolation, purification and partial characterization of viper venom inhibiting factor from the root extract of the Indian medicinal plant sarsaparilla (*Hemidesmus indicus* R. Br.). Toxicon,1994; 32(12): 1551–1557.
15. Chatterjee I, Chakravarthy AK, Gomes A. Antisnake venom activity of ethanolic seed extract of *Strychnos nux vomica* Linn. Indian Journal of Experimental Biology 2004; 42: 468–475.
16. Dassault Systèmes BIOVIA, Discovery Studio, Version 4.0, San Diego: Dassault Systèmes, 2016.
17. *https://pubchem.ncbi.nlm.nih.gov/compound/4-Methoxysalicylic_acid*
18. *https://pubchem.ncbi.nlm.nih.gov/compound/caffeic_acid*